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(54) Title: METHODS AND COMPOSITIONS FOR REGULATION OF 5-ALPHA REDUCTASE ACTIVITY

(57) Abstract

Compounds that inhibit 5α-reductase are provided. The compounds are used to treat prostate cancer, breast cancer, obesity, skin disorders and baldness.

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METHODS AND COMPOSITIONS FOR REGULATION OF 5-ALPHA REDUCTASE ACTIVITY

Technical Field of The Invention

The present invention relates generally to compounds, compositions and methods regulating the action and function of androgens and other steroid hormones by modulating the activity of steroid-reductases, including isozymes of 5α-reductases. More specifically, the present invention relates to the use of these compounds to regulate processes or treat disorders that are modulated by androgens or other steroid hormones or are caused by abnormal actions of androgens or other steroid hormones in cells or organs of animals, humans, plants, or microorganisms. This invention relates to the use of natural and synthetic flavanoids, catechols, curcumin-related substances, quinones, catechins and fatty acids and their analogues or derivatives as 5α-reductase isozyme inhibitors and as therapeutic agents. These compounds can also be used in promoting or modulating desirable production of specific products for commercial purposes.

Background of the Invention

In some of the androgen-sensitive organs, such as the prostate and skin, testosterone (T) is converted to a more active metabolite 5α-dihydrotestosterone (DHT) by 5α-reductase (Anderson and Liao, 1968; Bruchovsky and Wilson, 1968). Other substrates of 5α-reductases are also converted to reduce products that may have specific properties. Inhibition of 5α-reductase represents a unique approach for developing therapeutic methods for androgen-dependent diseases, such as benign prostatic hyperplasia, breast and prostatic cancer, skin disorders, seborrhea, common baldness, hirsutism, and hidradenitis suppurative. Various compounds have been shown to inhibit 5α-reductase activity (Liang and Liao, 1992; Hirsch et al., 1993; Russell and Wilson, 1994; Liao and Hiipakka, 1995). Finasteride (Proscar), a 5α-reductase inhibitor, lowers the level of DHT in serum and the prostate, reduces prostate volume and increase urinary flow in some patients (Stoner E. Finasteride Study Group, 1992). Certain aliphatic unsaturated fatty acids, such as γ-linolenic acid (Liang and Liao, 1992) and catechin-3-gallates (Liao

and Hiipakka, 1995), can inhibit 5α-reductase activity of liver and prostate of rats and humans in vitro.

5α-Reductase is found in many organs (Russell and Wilson, 1994; Hiipakka et al., 1993) including the sebaceous gland of hamsters (Takayasu and Adachi, 1972) and human hair follicles (Randall, 1994). Two 5α-reductase isozymes have been identified in rats and humans (Russell and Wilson, 1994). The type 1 isozyme predominates in rat tissues such as liver, kidney, brain, and lung, whereas the type 2 enzyme is more abundant in rat testis and epididymis. Both isozymes are found in skins of the neonate, but the type 1 isozyme is the major form expressed in the skin after puberty. The type 1 isozyme is also expressed in balding scalp. The possibility that the type 2 isozyme plays a unique role in skin and hair growth cannot be excluded. Finasteride, a 4-azasteroid, is a competitive inhibitor of 5α-reductases and has an affinity 30-fold higher for isozyme 2 than for isozyme 1 (Russell and Wilson, 1994). In contrast, the green tea catechins, epicatechin-3-gallate and epigallocatechin-3-gallate are more effective inhibitors of the type 1 enzyme and γ-linolenic acid inhibits both isozymes equally well (Liao and Hiipakka, 1995).

In the stumptail macaque, a monkey model of androgenic alopecia, finasteride given orally prevents frontal baldness (Diani et al, 1992). The paired hamster flank organs, one on each side of the costovertebral angle, are highly sensitive to androgen stimulation. Topical application of γ-linolenic acid suppresses only the androgen-dependent growth of the treated hamster flank organ without showing systemic effects on the contralateral flank organ and this effect is very likely due to local inhibition of 5α-reductase.

Uses of androgens known to the medical arts include, for example, treatment of hypogonadism and anemia. The abuse of androgens among athletes to enhance performance is well known. Androgens are also known to promote the development of benign prostatic hyperplasia (BPH), prostate cancer, baldness, acne, obesity and undesirable lipid and steroid profiles in blood and organs. Approximately 70% of males in the U.S. over the age of 50 have pathological evidence of BPH. Prostate cancer is the second leading cause of cancer death in

males in the U.S. Male-patterned baldness can start as early as the teens in genetically susceptible males, and it has been estimated to be present in 30% of Caucasian males at age 30, 40% of Caucasian males at age 40, and 50% of Caucasian males at age 50. Acne is the most common skin disorder treated by physicians. In women, hirsutism is one of the hallmarks of excessive androgen. The ovaries and the adrenal are the major sources of androgen in women.

In men, the major androgen circulating in the blood is testosterone. About 98% of the testosterone in blood is bound to serum proteins (high affinity finding to sex-steroid binding globulin and low affinity binding to albumin), with only 1-2% in free form. The albumin-bound testosterone, the binding of which is readily reversible, and the free form are considered to be bioavailable, and account for about 50% of total testosterone. Testosterone enters target cells apparently by diffusion. In the prostate, seminal vesicles, skin, and some other target organs, it is converted by a NADPH-dependent 5α-reductase to a more active metabolite, 5α-DHT. 5α-DHT then binds an androgen receptor (AR) in target organs. The 5α-DHT-receptor complexes interact with specific portions of the genome to regulate gene activities (Liao et al., 1989). Testosterone appears to bind to the same AR, but it has a lower affinity than 5α-DHT. In tissues such as muscle and testes, where 5α-reductase activity is low, testosterone may be the more active androgen.

The difference between testosterone and 5α-DHT activity in different androgen-responsive tissues is further suggested by findings in patients with 5α-reductase deficiency. Males with 5α-reductase deficiency are born with female-like external genitalia. When they reach puberty, their plasma levels of testosterone are normal or slightly elevated. Their muscle growth accelerates, the penis enlarges, voice deepens, and libido toward females develops. However, their prostates remain non-palpable, they have reduced body hair, and they do not develop acne or baldness.

The findings in 5α-reductase deficient patients suggest that inhibitors of 5α-reductase would be useful for the treatment of prostatic cancer, BPH, acne, baldness, and female hirsutism. Clinical observations and animal experiments have indicated that spermatogenesis, maintenance of libido, sexual behavior, and

feedback inhibition of gonadotropin secretion do not require the conversion of testosterone to 5α-DHT. This is in contrast to other hormonal therapies which abolish the actions of both testosterone and 5α-DHT.

Treatment of androgen-dependent skin and prostatic diseases by 5α-reductase inhibitors would be expected to produce fewer side effects than the presently available hormonal therapies. These include castration, estrogen therapy, high doses of superactive gonadotropin-releasing hormone such as Luprolide, and the use of competitive antiandrogens which inhibit AR binding of testosterone and 5α-DHT, such as flutamide, cyproterone acetate and spironolactone. The long term efficacy of competitive antiandrogens is also compromised by their block of the androgenic feedback inhibition of gonadotropin secretion. This increases testicular secretion of testosterone. The higher level of testosterone eventually overcomes the action of the antiandrogen.

Excessive 5α-DHT is implicated in certain androgen-dependent pathological conditions including BPH, acne, male-pattern baldness, and female idiopathic hirsutism. It has been shown that 5α-reductase activity is reported to be higher in hair follicles from the scalp of balding men than that of non-balding men.

Since treatments of androgen-dependent skin and prostatic diseases by 5α-reductase inhibitors can produce fewer side effects than the hormonal therapies which indiscriminately inhibit all androgen actions, it is desirable to provide different types of 5α-reductase inhibitors.

Brief Summary Of The Invention

The present invention relates generally to the utilization of certain compounds for the control of androgen activity in target organs and cells through the modulation of a 5α-reductase activity. In certain aspects, these compounds are employed to repress androgenic activity by inhibiting the formation and availability of active androgen in target cells. Consequently, the present invention is useful for the treatment of a wide variety of conditions including, but not limited to, the treatment of prostatic hyperplasia, prostatic cancer, hirsutism, acne, male pattern baldness, seborrhea, and other diseases related to androgen hyperactivity. Several of these compounds have been shown to effectively decrease body weight, and in

some cases, to decrease the weight of an androgen-dependent body organ, such as the prostate and other organs. The effectiveness of these compounds may be dependent also on their action on other mechanisms involved in angiogenesis, cell-cell interaction, and on their interaction with various components of organs and cells.

Compounds useful in the practice of the present invention include various isomers of saturated and unsaturated fatty acids, natural and synthetic analogues, and derivatives from which these fatty acids can be generated as well as the metabolites and oxidation products of these fatty acids. The use of these and other fatty acids and their derivatives is also contemplated. Also useful are catechin compounds, particularly, catechins that are structurally similar to epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). EGCG has an additional hydroxyl group on the epicatechin gallate molecule, which has been found to be surprisingly active in modulating several 5α-reductase mediated processes. EGCG derivatives having such an additional OH group on the altering ECG molecule were shown to be active in inducing body weight loss and particularly in reducing the size of androgen sensitive organs such as preputial glands, ventral prostate, dorsolateral prostate, coagulating glands, seminal vesicles, human prostate tumors, and breast tumors in nude mice.

By analogy with the fatty acid compounds, certain active catechin gallates may not enter target cells easily. However, esterification of hydroxyl groups on the inhibitor compounds should enhance the ability of these compounds to enter the target cells. Once inside the cells, esters would be readily hydrolyzed by esterase to alcohols that can inhibit 5α-reductases (Williams, 1985).

In more particular aspects of the invention, the inventors have discovered that certain catechins, particularly EGCG, can be administered to promote body weight loss that differentially affects overall body weight and prostate weight loss. In particular examples, it was shown that for a certain percentage of overall body weight loss, prostate weight loss was percentage-wise more than three times as much. The loss in body weight and the organ weight are likely due to EGCG interference of a common step in the pathway controlling body weight and the

organ weight gain. EGCG and related compounds may interact and interfere with a receptor macromolecule (probably containing a protein) that modulates specific lipid synthesis and accumulation. Lipids can modulate gene expression, cell development and differentiation, and organ growth. Specific interference of lipid metabolism in the cells and organs may control the growth of the organs, in particular, prostate, sebaceous, preputial and other secretory organs. In certain applications, it is expected that benign or abnormal growth or cancer of these organs may be treated or even prevented by administration of catechin related compounds.

It has been demonstrated that catechin compounds will arrest or reduce human prostate and breast cancer cell growth. The effectiveness of catechin compounds was shown to be dependent on the methods by which these compounds were administered to the experimental animals. Intraperitoneal application was much more effective than oral administration. It is expected that direct application to the organs, such as the prostate, will be very effective. EGCG was surprisingly effective in suppressing and even reducing the size of human prostate and breast tumors in animal models. The effect was illustrated with EGCG; however, structurally similar catechin compounds are also effective, particularly those that are structurally similar to EGCG in having at least one additional hydroxyl group as compared with ECG. Thus, the EGCG species that contain eight hydroxyl groups is significantly more effective in reducing body weight than is ECG, which contains seven hydroxyl groups. Compounds of this general structure are expected to be particularly effective in chemoprevention and chemotherapy of human prostate cancer. Compounds having a part of structure similar to a part of structure of EGCG are also expected to be effective.

Compounds can be used as antiandrogenic agents through topical or systemic application. A preparation for this purpose can include a carrier, a protectant, an antioxidant (such as vitamin C or E, and various catechins and polyphenols), and other pharmaceutical and pharmacological agents. It is also expected that such compounds can be used in a delivery system (oral, local application, injection, or implantation) involving molecular recognition through

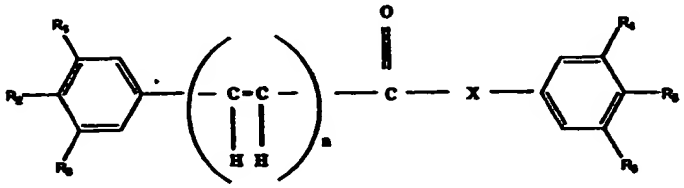
which the compounds are delivered to target sites. Such a delivery system may involve, among other methods, liposome techniques or immunological devices.

Natural or synthetic chemicals that can modulate the production or cellular action of receptors and macromolecules are useful in the treatment of abnormalities such as obesity, BPH, prostate cancer, skin diseases, baldness, breast tumors, and hirsutism, which are related to lipid synthesis, body weight, and/or androgen function.

Animal models can be used to demonstrate the effectiveness of compounds on a variety of cancers. For example, Shionogi tumor and other tumors can be studied in male rats. Human breast and prostate cancer cell growth can be studied in nude mice. Alternatively, rodent breast tumors induced by carcinogens and other cancers induced in transgenic mice or Dunning tumor in rats can be similarly analyzed for their chemotherapy by EGCG and related compounds.

The use of compounds disclosed in the present invention, or in natural therapeutically effective amounts of pharmaceutical compositions containing one or more of the compounds, in some cases in combination with other therapeutic agents and carriers, or in natural or synthetic products, is appropriate in the treatment of various disorders. These disorders include, but are not necessarily limited to, those conditions wherein excessive androgenic activities have been implicated, for example, male pattern baldness, female hirsutism, skin disorders, BPH, cancers of prostate, breast, skin and other organs.

The present invention is also directed to novel compounds. These compounds have the formula:



I

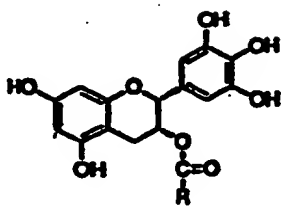
where x is $-NHCH_2CH_2-$ or -CH=CH-;

R_1, R_2 and R_3 each may be -H, -OH or $-OCH_3$, provided that only one of R_1, R_2, and R_3 may be -H;

R_4, R_5 and R_6 each may be -H, -OH, -OCH_3 or -$N(CH_3)_2$, provided that only one of R_4, R_5 and R_6 may be -H; and

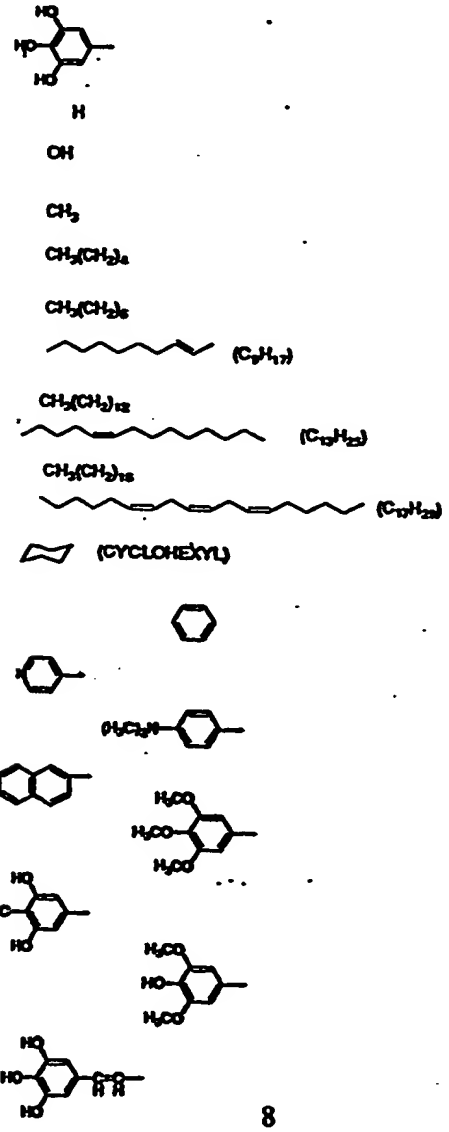
n is 0 or 1.

and the formula:



II

where R is



All of the compositions and methods disclosed and claimed herein can be made without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Brief Description Of The Drawings

In the drawings, which form a portion of the specification:

Fig. 1 shows the structure of flavanoid compounds of the present invention.

Fig. 2 shows the structure of catechol compounds of the present invention.

Fig. 3 shows the structure of curcumin and related compounds of the present invention.

Fig. 4. shows quinones of the present invention.

Fig. 5 shows epigallocatechin derivative compounds of the present invention.

Fig. 6 shows the generic formula of the epigallocatechin derivatives of the present invention;

Fig. 7 shows the generic formula of gallates useful in the present invention;

Fig. 8 shows the generic formula of curcumin derivatives useful in the present invention;

Fig. 9 shows the generic formula of quinones and catechols useful in the present invention.

Fig. 10 shows fatty acids of the present invention.

Detailed Description Of The Invention

I. 5α-Reductase Activity

The present invention is concerned with methods of inhibiting 5α-reductase, which include subjecting a cell to an effective concentration of a 5α-reductase inhibitor such as one of the compounds disclosed herein. It is believed that the use of such inhibitors to block abnormal androgen action will serve to treat cancer in conjunction with other anti-cancer agents, chemotherapy, resection, radiation therapy, and the like. The compounds of this invention, besides acting as 5α-reductase inhibitors, may have other effects that can lead to antitumor activity or to suppress abnormal growth of prostate or other organs.

In mammalian cells, 5α-reductase is very tightly associated with intracellular membranes, including the membrane of the endoplasmic reticulum and contiguous nuclear membranes. Attempts to solubilize and purify active 5α-reductase have not been very successful. The assay of 5α-reductase activity, therefore, is performed by measuring the rate of conversion of testosterone to 5α-DHT by whole cells or by microsomal and nuclear preparations in the presence of NADPH (enzymatic assay). Alternatively, the 5α-reductase activity can be reliably assayed by following NADPH-dependent noncovalent binding of a potent radioactive inhibitor, such as [^{3}H]4-MA ([^{3}H]4-MA-binding assay), which strongly competes with testosterone for binding to the reductase. The results of the two assays correlate very well when microsomal preparations from different organs or animals are used for comparison.

A. [^{3}H]4-MA Binding Assay For 5α-Reductase

Briefly, the binding assay solution, in a final volume of 0.15 ml, contains microsomes (2-20 μg of protein), 0.07 μCi of [^{3}H]4-MA, 0.1 mM-NADPH, 1 mM-dithiothreitol and 50 mM-potassium phosphate, pH 7.0, with or without the indicated amount of a lipid or an inhibitor preparation. Lipids are dissolved in ethanol and added in 1-5μl volumes. Control tubes receive the same amount of ethanol. After incubation at 0° C. for 1 hour, the [^{3}H]4-MA bound to microsomes is determined by collecting microsomes on a Whatman glass fibre filter

and washing with 10 ml of 20 mM-potassium phosphate, pH 7.0, containing 0.01% CHAPS to remove unbound [^{3}H]4-MA.

B. Assays Of The Enzymatic Activity Of Microsomal 5α-Reductase

The standard reaction mixture, in a final volume of 0.15 ml, contains microsomes, 1 μCi of [^{3}H]testosterone, 0.5-3.0 μM non-radioactive testosterone, 0.1mM-NADPH, 1mM-dithiothreitol and 50 mM-potassium phosphate, pH 7.0, with or without the indicated amount of a lipid or an inhibitor preparation. The reaction is started by the addition of microsomes and the incubation is carried out at 37° C. for 15 minutes. Steroids are extracted and separated by thin layer chromatography. Radioactive steroids are located by fluorography and the amount of radioactivity present determined by scintillation counting. The 5α-reductase activity was measured by analyzing the extent of the conversion of [^{3}H]testosterone to [^{3}H]5α-DHT.

C. Sources of 5α-Reductase Activity

Microsomes are prepared at 4° C. from a buffered 0.32 M-sucrose homogenate of human liver and from the livers of adult Sprague-Dawley female rats by differential centrifugation, and are used in the assay of 5α-reductase activity. In some experiments, microsomes are solubilized with 0.1% polyoxyethylene ether W-1, except for the substitution of polyoxyethylene ether W-1 for Lubrolx-WX.

Cells genetically engineered to express specific types of 5α-reductase isozymes can also be used as sources of 5α-reductase activity. Intact cells containing 5α-reductase, their microsomes, or nuclear preparations can also be used to screen 5α-reductase inhibitors.

II. Prostate And Breast Cancer

A compound of this invention can be used to treat breast or prostate cancer. The effectiveness of such compounds against prostate and breast cancer can be determined either on isolated cell lines derived from such cancer tissues or in animals demonstrating these forms of cancer. By way of example, human prostate cancer PC-3 cells are grown in culture medium. About one million cells are injected into male nude mice and the growth of tumors followed. Within two

weeks, the tumor grows to about 100 mm^3. Three tumor bearing mice are injected with a test compound each day.

III. Organ And Body Weight Loss

A compound of this invention can be used to decrease organ and body weight. The compounds thus have use in treating obesity. The effectiveness of a compound can be determined using well-known animal models.

By way of example, male Sprague-Dawley rats (body weight 180 g±10g) are used. Compounds are intraperitoneally injected into rats in one group each day for 7 days. Rats in the control group receive 0.1 ml 30% ethanol. Body and organ weights are determined.

IV. Skin Disorders

The inventors sought an inhibitor of 5α-reductase that would be active topically and inactive systemically; such an agent would be ideal for treatment of androgen-dependent dermatological disorders. In this study, inhibition of androgen action by topical administration of γ-LA in hamster flank organs is investigated. Especially useful in the evaluation of the effects of these compounds on skin cells or sebaceous glands is the hamster flank organ (Frost and Gomez, 1972). The paired flank organs, one on each side of the costovertebral angle, are highly sensitive to androgen stimulation. The androgen sensitive structures in the flank organ include dermal melanocytes, sebaceous glands, and hair follicles (Hamilton and Montagna, 1950). This animal model has been widely used for testing androgenic (Hamilton and Montagna, 1950; Frost et al., 1973) and antiandrogenic compounds (Voigt and Hsia, 1973; Weissmann et al., 1985; Chakrabarty et al., 1980). The unique advantage of this animal model is that a testing compound can be applied topically to only one of the flank organs and the effect observed on both organs. If the test compound has only a local effect, then only the treated flank organ is affected. However, if the effect is systemic, then both flank organs are affected.

Pre-pubertal male Syrian golden hamsters, castrated at 4 weeks old, are obtained from Harlan Sprague-Dawley Co. (Madison, WI). Each animal is

maintained individually in a plastic cage on rodent chow (Purina) and water ad libitum on a 12 hour light/12 hour dark cycle.

One to two weeks after castration, the hair on the lower back of each animal is clipped with an electric hair clipper and then shaved weekly to expose the flank organs. The animals are divided into treatment groups. A treatment solution (5 µl) is applied topically to the right flank organ once a day using a Pipetteman and a polypropylene disposable tip. Unless specified, the left flank organ is not treated. The treatment solution contains either (a) ethanol alone (vehicle and control), or (b) a test compound. The flank organ was wiped with an alcohol pad to remove residual compound before each treatment. At the end of each experiment (17-25 days), the animals were sacrificed by either suffocation with CO_2 gas or with an intraperitoneal injection of an overdose of phenobarbital (64.8 mg/ml/animal). The flank organs, both the treated and untreated sides, are evaluated to determine the effect of these treatments on the growth of the pigmented macule and the sebaceous glands. The body weight of each animal is recorded before and after treatment.

Treatment Of Animals

Male hamsters, 4 weeks old, are castrated and are kept on a longer light period (16 hours light/8 hours dark cycle) to insure maximum stimulation of sexual characteristics (Luderschmidt et al., 1984). Flank organs, left and right, were treated topically with 5 µl ethanol containing 0.5 µg or 1 µg testosterone daily. Animals are divided into groups of 4-5 hamsters. The right flank organ is also treated daily with 5 µl solution containing vehicle (ethanol) alone or test compound (1 or 2 mg) for 18 days. The left flank organ of all animals receives the same volume of vehicle.

the lengths of the long axis and the short axis of the pigmented spot (pigmented macule) are measured using a caliper with digital display (Digimatic, Mitutoyo Corp., Japan). The product (long axis x short axis, mm^2) is used as an index of the surface area (Wuest and Lucky, 1989).

The flank organ treated with test compound becomes elevated and palpable. The length of the long axis and short axis of the elevated mass are measured with a caliper. The product of the long axis x short axis (mm^2) was used as an index of the

areas of the sebaceous gland, which correlates with the volume of the sebaceous glands.

V. **Baldness**

Topical Effects Of Compounds On Hair Loss And Growth

The stumptail macaque monkey develops baldness in a pattern resembling human androgenetic alopecia. The balding process begins shortly after puberty (approximately 4 years of age). This occurs in nearly 100% of the animals, males and females, and is androgen dependent. This is a useful animal model for human androgenetic alopecia and is contemplated to be useful in demonstrating the effects of polyunsaturated fatty acids on hair loss. The following describes a protocol for testing.

Male stumptail macaques (4 years of age) are divided into groups of 3 to 5 animals. A defined area of the scalp involving the frontal and vertex areas is marked, e.g., by tattoo. Hairs in the marked area are shaved. The solutions of a test compound in different dosages and combinations are evenly applied to the shaved areas once or twice a day. Control animals receive the same volume of the solvent (e.g., ethanol or other organic solvent, or a cream). The same area of the scalp is shaved every 4 to 6 weeks and the weights of hairs shaved are determined. The treatments may last for 6 months to 2 years. 4-MA (17-N,N-diethylcarbamoyl-4-methyl-4-aza-5-androstan-3-one), a 5α-reductase inhibitor known to prevent baldness in this animal is included as a positive control. Biopsies of the scalp (4mm punch) are obtained before and at the end of the treatments. The specimens are analyzed for 5α-reductase activity and examined histologically for evidence of alopecia.

VI. **Effects Of Compounds On Sebum Productions In A Human Model**

Topical antiandrogenic activity of several fatty acids and catechins is first evaluated in the hamster flank organ assay or the rat assay. To further confirm the effectiveness of antiandrogenic compounds and suitability for human use, tests are performed on a human male subject. The ideal compounds for human treatment are those that are topically and locally active but do not show systemic antiandrogenic activity, especially in the cases involving young males.

Determination Of Forehead Sebum Production

A male volunteer is used to test and analyze sebum production from the forehead region. The forehead is washed thoroughly with soap twice and then cleaned with 70% isopropyl alcohol twice. Sebum production is measured 30 to 60 minutes later with a sebum meter tape probe (7 mm x 8 mm) covering 56 mm^2 area in each measurement. Ten measurements are made within the 4 cm square area ($16cm^2$) located at the middle of the left or right side forehead between the eyebrow and the hair line.

The sebum meter detects the difference in the transparency of the tape before and after the tape was placed on the forehead for 30 seconds and expresses the difference in an arbitrary number (S-value) between 0 to 300 (or higher). S-values of sebum accumulated on the foreheads of men are usually 200 to 300. Skin surface on hands usually shows a very low number (5 to 20). The S-value for forehead immediately after washing is less than 5. For men, the S-value gradually increases to about 50 within 30 minutes after washing and reaches 100 to 200 in 45 minutes to 55 minutes.

To determine the rate of sebum production, the left and the right forehead areas are measured alternatively and each time at the comparable areas on the two sides. Ten measurements on each side (i.e., 20 measurements for two sides) take about 15-20 minutes and the sebum-values likely range between 30 to 200. The S-values can differ considerably at different areas of the forehead and could be influenced by environmental, including weather, diet, and physiological, conditions. However, the ratio of the total S-value (the sum of 10 measurements) for the left and the total S-value for the right forehead is constant. Therefore, compounds applied to the left forehead that reduce the L/R ratio to lower than 1.1 are considered as topically active agents for suppression of sebum production.

VII. Pharmaceutical Compositions

Aqueous compositions of the present invention comprise an effective amount of the 5α-reductase inhibitory agent dissolved or dispersed in a pharmaceutically acceptable aqueous medium. The phrase "pharmaceutically

acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

The preparation of an aqueous composition that contains such an inhibitory compound as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectable, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

The pharmaceutical compositions disclosed herein may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or may be compressed into tablets, or they may be formulated for controlled release, such as a transdermic and osmotic pressure device, injectable device, and implantable device, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the compositions and preparations may, of course, be varied and may conveniently be 100% (application of pure compounds). The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, such as gum tragacanth, acacia, corn starch or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin; or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compounds; sucrose, as a sweetening agent, methyl and propylparabens as preservatives; a dye and flavoring, such as cherry or orange flavor. Of course, any

material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic. In addition, the active compounds may be incorporated into sustained-release preparations and formulations.

The active compounds may also be administered parenterally, intravenously, or intraperitoneally. Solutions of the active compounds as a free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquified polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such a lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for

pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

For oral administration the composition may be incorporated with excipients and used in the form of non-ingestible mouthwashes and dentifrices. A mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an antiseptic wash containing sodium borate, glycerin and potassium bicarbonate. The active ingredient may also be dispersed in dentifrices, including: gels, pastes, powders and slurries. The active ingredient may be added in a therapeutically effective amount to a paste dentifrice that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants.

The composition can be formulated in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formation, solutions will be administered in a manner compatible with the dosage formulation and in such a manner as it therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

In other embodiments, one may desire a topical application of compositions disclosed herein. Such compositions may be formulated in creams, lotions, solutions, or in solid form depending upon the particular application. The formulation of pharmaceutically acceptable vehicles for topical administration is well known to one of skill in the art (see, i.e., "Remington's Pharmaceuticals

Sciences", 15th Edition). Variation of the dosage of the compositions disclosed herein, will necessarily depend upon the particular subject, and the nature of the condition(s) being treated.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermic or intravenous fluid or injected at the proposed site of infusion, (see, for example, "Remington's Pharmaceutical Sciences", 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

EXAMPLE

Assays For Candidate Substances

Expression Of Human 5α-Reductases. For the preparation of rat 1A cells expressing different types of human 5α-reductases, cDNAs for the human type 1 and 2 5α-reductases were isolated from human prostate λgt11 and PC-3 cell λZAP II cDNA libraries using the published sequence of the 5α-reductases, PCR and standard library screening techniques. The type 1 and 2 cDNAs were subcloned into the retroviral expression vector pMV7 and high titer stocks of virus containing the type 1 and 2 cDNAs were generated using the packaging cells BOSC 23 293. Rat 1A cells were infected with virus and cells containing integrated retrovirus were selected for G418 resistance (Brown and Scott, 1987).

Assay Of 5α-Reductase. Microsomes were prepared from rat 1A cells expressing specific types of human 5α-reductase. The enzymic assay was based on

the measurement of 5α-DHT production from testosterone in the presence of microsomes prepared from rat 1A cells containing either the type 1 or type 2 human 5α-reductase. The amount of labeled testosterone and dihydrotestosterone in extracts was determined by thin layer chromatography and scanning on a AMBIS radioanalytic scanner. The concentration of test compound inhibiting the conversion of testosterone to dihydrotestosterone by 50% (IC50) was determined by interpolation between appropriate data points.

Inhibition Of 5α-Reductase Activity.

Previously, we showed that two natural products, the green tea catechins, epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) and unsaturated fatty acids were inhibitors of human 5α-reductase. A structure-activity relationship study was initiated to explore the structural requirements for this activity. Data for this study are summarized in Tables 1-7 and Figs. 1-6.

1. **FLAVANOIDS**

A variety of naturally occurring flavanoids with structures related to the tea catechins were tested (Fig. 1, Table 1).

TABLE 1

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Epicatechin Gallate	11	100	60	83
2. Epigallocatechin Gallate	15	99	74	74
3. Myricetin	23	96	>100	31
4. Quercitin	23	96	>100	14
5. Baicalein	29	79	99	51
6. Fisetin	57	97	>100	4
7. Biochanin a	>100	50	17	74
8. Kaempferol	>100	22	12	62
9. Flavone	>100	20	>100	(-52)
10. Genistein	>100	16	23	76

11. Epigallocatechin	>100	15	>100	3
12. Epicatechin	>100	14	>100	4
13. Morin	>100	6	>100	33
14. Alpha-napthoflavone	>100	6	>100	(-13)
15. Taxifolin	>100	5	>100	5
16. Rutin	>100	4	>100	0
17. Daidzein	>100	3	29	69
18. Beta-napthoflavone	>100	3	>100	4
19. Chrysin	>100	2	>100	1

The tea catechins, ECG and EGCG, had the highest activity of the tested flavanoids and were better inhibitors of the type 1 (HRED1) than the type 2 (HRED2) isoenzyme of 5α-reductase. The tea catechins epicatechin (EC) and epigallocatechin (EGC) had little activity. Four flavanoids, myricetin, quercitin, baicalein and fisetin had significant (IC50<100 μM) activity and were more active against the type 1 than the type 2 isoenzyme. Biochanin A, kaempferol, genistein, and diadzein were effective inhibitors of the type 2 but not type 1 isoenzyme. Comparison of the activities of chrysin, kaempferol, morin, myricetin, and quercitin indicate the importance of B-ring hydroxyl groups, especially in a catechol or pyrogallol configuration, and perhaps the importance of the hydroxyl at position 3 for activity against the type 1 isozyme. Rutin, the 3-rutinose glycoside of quercitin was ineffective against either isoenzyme (IC50>100 μM). The inactivity of rutin compared to quercitin is either due to the presence of the oligosaccharide rutinose (perhaps due to steric hindrance) or modification of the hydroxyl at position 3. Taxifolin, a flavanone, was ineffective against either isozyme (IC50>100 μM). The weak activity of taxifolin is most likely due to the absence of the 2,3-unsaturated bond when its activity is compared to the structurally related quercitin. When tested for inhibitory activity in whole cells, most flavanoids showed little or no activity against the type 1 isoenzyme, perhaps indicating limited penetration of these polyhydroxy compounds across the cell membrane. In contrast to the results with the type 1 enzyme, four flavanoids, biochanin A, diadzein, kaempferol and

genistein had significant inhibitory activity against the type 2 isoenzyme in the whole cell assay. The most active of these, biochanin A and diadzein, have only two and three free hydroxyl groups, respectively, and so may penetrate cells easier than other flavanoids.

2. CATECHOLS

5α-reductase inhibition studied with the flavanoids indicated the potential importance of catechol and pyrogallol moieties for high inhibitory activity. Therefore, a series of compounds with catechol groups was surveyed for activity (Table 2, Fig. 2).

TABLE 2

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Anthrarobin	4	99	50	97
2. Bromopyrogallol Red	7	98	84	58
3. Gossypol	7	99	21	99
4. Pyrogallol Red	15	97	>100	27
5. Nordihydrogaiaretic Acid	19	99	50	80
6. Caffeic Acid Phenethyl Ester	26	97	>100	36
7. Octyl Gallate	27	99	58	90
8. Purpurogallin	30	81	>100	31
9. Hydroxydopamine	42	69	>100	41
10. Dodecylgallate	43	88	>100	36
11. Pyrocatechol Violet	48	85	100	47
12. Pyrogallol	70	60	>100	28
13. Hematoxylin	83	59	>100	38
14. HZIV-82	>100	43	>100	0
15. Cnc	>100	42	>100	(-75)
16. HZIV 90	>100	23	>100	13
17. Caffeic Acid	>100	13	>100	8

18. HZIV 275	>100	10	>100	6
19. Exculetin	>100	7	>100	13
20. Ellagic Acid	>100	7	>100	9
21. Catechol	>100	5	>100	0
22. Methylgallate	>100	5	>100	3
23. Fraxetin	>100	2	>100	8
24. Propylgallate	>100	0	>100	0

Thirteen of the 24 compounds listed had IC50's below 100 μM. All were more active against the type 1 than type 2 isoenzyme. Six of these compounds, anthrarobin, dodecyl gallate, gossypol, octyl gallate, caffeic acid phenethyl ester and nordihydroguaiaretic acid were active in whole cell assays (Table 7, below). Anthrarobin was much more effective against the type 1 than type 2 isoenzyme; whereas, the other five inhibitors were equally effective inhibitors of both isoenzymes. The synthetic compound HZIV 82 showed little activity in the cell-free assay, but was very active in the whole cell assay with specificity for the type 1 isoenzyme.

3. CURCUMIN AND RELATED COMPOUNDS

Curcumin was a very effective inhibitor of either the type 1 or type 2 isoenzyme (Table 3, Fig. 3).

TABLE 3

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Curcumin	3	95	5	87
2. Tetrahydrocurcumin	80	56	29	73
3. Demethoxy-tetrahydrocurcumin	>100	23	>100	42
4. 4-hydroxy-3-methoxy-cinnamaldehyde	>100	10	>100	(-60)
5. Coniferol	>100	10	100	49

6. 4-(4-hydroxy-3-methoxyphenol)-3-buten-2-one	>100	3	>100	4
7. Ferulic Acid	>100	0	>100	18
8. Capsaicin	>100	0	>100	8
9. Eugenol	>100	0	100	50

Commercially available curcumin was chemically reduced with Pt/H2 and the products, tetrahydrocurcumin and demethoxytetrahydrocurcumin, had much less activity than curcumin. However, tetrahydrocurcumin (HZIV 81-2), which is colorless compared to the bright yellow curcumin, had significant activity in the whole cell assay. The structurally related compounds 4-(4-hydroxy-3-methoxyphenol)-3-buten-2-one, ferulic acid, capsaicin, eugenol and coniferyl alcohol had little inhibitor activity (IC50>100 μM) against either isoenzyme highlighting the importance of the diferulolyl structure for activity against 5α-reductase. Nordihydroguaiaretic acid (NDGA) was also an effective inhibitor of the type 1 (IC50=19 μM) and type 2 (IC50=50 μM) isozymes in cell-free and whole cell assays, but less so than curcumin.

4. QUINONES

A variety of quinones were tested for activity against 5α-reductase (Table 4, Fig. 4).

TABLE 4

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Purpurin	2	95	>100	20
2. Alizarin	3	95	100	54
3. Anthrarobin	4	99	50	97
4. Menadione	6	77	5	81
5. Coenzyme q	12	77	22	81
6. 2,5-dichloroindophenol	15	78	17	97
7. Alizarin Red 5	30	91	>100	8

8. Anthrarufin	40	67	>100	13
9. Anthrarufin	40	67	>100	13
10. Lapachol	>100	30	>100	9
11. Anthraflavic Acid	>100	27	>100	22
12. Quinizarin	>100	26	>100	7
13. T-butylhydroxyquinone	>100	19	>100	4
14. Anthraquinone	>100	6	>100	9

The naturally occurring anthraquinone, alizarin, was a very effective inhibitor of the type 1 but not type 2 isozymes. Alizarin Red S, which is a water soluble sulfate derivative of alizarin had little activity (IC50s>100 μM) against either isoenzyme. The charged sulfate group may prevent interaction with membrane bound 5α-reductase. Purpurin, which has an additional hydroxyl compared to alizarin, had inhibitory activity similar to alizarin. Anthraflavic acid, anthrarufin and quinizarin, which are structural isomers of alizarin without adjacent hydroxyl groups, had much less activity, emphasizing the importance of the catechol moiety for potent inhibitory activity of this class of anthroquinones. Anthraquinone was not an effective inhibitor (IC50>100 μM). Menadione, coenzyme Q, and 2,6-dichloroindophenol were potent cell-free inhibitors of boh isoenzymes. The compounds participate in quinone reductase reactions and may deplete NADPH causing the observed inhibition. In the whole cell assay, alizarin was a very effective inhibitor of the type 1 isoenzyme and menadione had moderate activity.

5. <u>EPIGALLOCATECHIN DERIVATIVES</u>

The high inhibitory activity of EGCG in a cell-free assay but low in the whole cell assay led us to design and synthesize a series of derivatives of EGC to enhance activity in the whole cell assay (Table 5, Fig. 5).

TABLE 5

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. EGCG	12	99	73	76
2. HZIV 160	29	99	76	96
3. HZIV 134	20	99	67	94
4. HZIV 92	23	98	>100	45
5. HZIV 120	23	99	66	97
6. HZIV 142	25	97	63	93
7. HZIV 68	29	93	99	51
8. HZIV 75	29	97	>100	21
9. HZIV 166	30	98	78	74
10. HZIV 63	311	94	>100	20
11. HZIV 169	47	90	>100	39
12. HZIV 74	48	85	>100	24
13. HZIV 144	49	88	>100	38
14. HZIV 168	49	98	73	92
15. HZIV 166	59	95	71	84
16. BGC	62	61	>100	30
17. HZIV 107	98	52	>100	39
18. HZIV 145	>100	35	>100	8
19. HZIV 148	>100	31	>100	0
20. HZIV 109	>100	17	>100	0

The studies showed that derivatization of the hydroxyl groups of EGCG with methyl or acetate groups leads to the loss of inhibitory activity in the cell-free assay and no enhancement of the lower whole cell assay inhibitory activity. We, therefore, limited structure-activity studies to changes in the gallate ester moiety of EGCG to enhance inhibitory activity in the whole cell assay. Twenty of these

structural changes are summarized in Table 5. The most significant structural change leading to activity in the whole cell assay was introduction of fatty acid ester in place of the gallic acid group of EGCG. In particular, fatty acids with some degree of unsaturation had good inhibitory activity against both isoenzymes of 5α-reductase in the whole cell assay. The most potent of these derivatives was one with γ-linolenic acid esterified to the 3-hydroxyl of EGC. Certain fatty acids with a single unsaturated bond were also active. For example, HZIV 160, the myristoleic ester of EGC was effective in both assay systems. Fatty acids with less unsaturation are less susceptible to oxidation and so may be more suitable modifying agents.

TABLE 6

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Gamma-Linolenic Acid C18:3 CIS 6.9,12	5	99	11	89
2. Crocetin	7	70@30	>100	20@30
3. Alpha-Linolenic Acid C18:3 CIS 9,12,15	8	99	9	84
4. Linoleic Acid C18:2 CIS 9,12	9	99	19	85
5. Oleic Acid C18:1 CIS 9	10	99	42	86
6. Conjugated Octadecadienonic Acid	10	99	30	81
7. 5,8,11,14-Eocpsatetraynoic Acid	15	97	3	81
8. Stearic Acid C18:0	27	71	>100	35

TABLE 7

Whole Cell Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. HZIV 82	3	79	>100	15
2. Dodecylgallate	3	99	7	98
3. Anthrarobin	6	91	>100	31
4. Alizarin	6	75	>100	27
5. Gossypol	7	100	6	99
6. HZIV 160	7	99	8	98
7. Octyl Gallate	7	99	18	94
8. Caffeic Acid Phenethyl Ester	8	99	7	98
9. HZIV 142	8	99	14	98
10. Curcumin	9	99	7	99
11. Nordihydroguaiaretic Acid	19	99	22	99
12. HZIV 165	28	97	32	98
13. HZIV 168	28	93	41	94
14. HZIV 81-2	36	81	7	92
15. HZIV 148	42	90	74	81
16. HZIV 75	43	83	62	72
17. HZIV 120	49	97	57	96
18. Menadione	51	82	79	62
19. HZIV 166	58	89	72	83
20. Biochanin A	64	64	5	93
21. HZIV 92	64	94	80	62
22. Kaempferol	79	60	20	85
23. Daidzein	10	13	7	89
24. Baicalein	>100	24	>100	4

25. Fisetin	>100	42	>100	27
26. EGCG	>100	11	>100	5
27. Myricetin	>100	11	>100	11
28. Purpurin	>100	47	>100	7
29. Quercetin	>100	15	>100	29
30. Alizarin Red S	>100	28	>100	1
31. Genistein	>100	22	20	89
32. HZIV 123	>100	48	>100	8
33. HZIV 107	>100	23	>100	2
34. Catechol	>100	9	>100	3
35. Daidzein	>100	9	58	87
36. Pyrogallol	>100	7	>100	15
37. EC	>100	0	>100	1
38. EGC	>100	15	>100	1
39. ECG	>100	0	>100	0
40. EGCG	>100	6	>100	0
41. HZIV 90	>100	34	>100	14
42. HZIV 63	>100	12	>100	7
43. HZIV 68	>100	40	>100	34
44. HZIV 144	>100	12	>100	7
45. HZIV 81-3	>100	28	19	80
46. HZIV 145	>100	8	>100	9
47. Methyl Gallate	>100	0	>100	0
48. Propyl Gallate	>100	5	>100	0
49. Isopropyl Gallate	>100	0	>100	0
50. Gallic Acid	>100	13	>100	0
51. Pyrogallol	>100	5	>100	6
52. HZIV 169	>100	10	>100	0
53.Gamma-Linolenic	22	91	20	86
54. Etya	22	67	2	86

55. Alpha-Linolenic	29	82	23	86
56. Linoleic	40	78	25	77
57. Oleic Acid	83	58	>100	45
58. Stearic Acid	>100	10	>100	23
59. Alpha-Linolenic Acid Me Ester	>100	24	>100	18
60. Gamma-Linolenic Acid Chol Ester	>100	12	>100	11
61. Gamma-Linolenic Acid Me Ester	>100	49	>100	26

As found previously in our lab, the greater the degree of unsaturation, the better the inhibitory activity of the fatty acid. Since unsaturated fatty acids are easily prone to oxidation which may comprise their usefulness, we examined some unsaturated fatty acids less prone to oxidation. The synthetic fatty acids, conjugated octadecadienoic acid (CODA) (cis or trans- 9,11 or 10,12 octadecadienoic acid) and 5, 8,11,14-eicosatetraynoic acid (ETYA), were good inhibitors of both isoenzymes. CODA and ETYA had IC50s of 10 and 15 (type 1) and 30 and 3 (type 2) μM, respectively. The naturally occurring fatty acid, γ-linolenic acid, has IC50 of 3 μM for both isoenzymes. Fatty acids such as ETYA may be useful for derivatizing other 5α-reductase inhibitors to enhance cellular uptake and promote in vivo activity of 5α-reductase inhibitors. Methyl and cholesterol esters of γ-linolenic acid had little activity in the whole cell assay (TABLE 7) and so the activity of EGC esterified to γ-linolenic acid is unlikely due to intracellular hydrolysis of these esters.

Active 5α-reductase inhibitors shown in Tables 1-7 are polyphenols or their derivatives and are easily oxidized or hydrolyzed within several hours to several days, especially in the presence of air or oxygen and at a pH above 7.0. We have found that these compounds are more stable to oxidation or hydrolysis by maintaining the pH of the solutions of these compounds at a pH below 7.0. More than 80% of the oxidation of hydrolysis can be prevented by the addition of an

inorganic acid, such as hydrochloric acid, sulfuric acid, or phosphoric acid, or an organic acid, such as citric acid or acetic acid.

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24. Williams, Clin. Pharmacokinetics, 10: 392-403, 1985.
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WHAT IS CLAIMED IS:

1. A process of inhibiting 5α-reductase activity comprising the step of exposing 5α-reductase to an effective inhibiting amount of a compound of any of Tables 1-7.

2. A process of treating prostate cancer in a subject in need of such treatment comprising the step of administering to the subject an effective therapeutic amount of a compound of any of Tables 1-7.

3. A process of treating breast cancer in a subject in need of such treatment comprising the step of administering to the subject an effective therapeutic amount of a compound of any of Tables 1-7.

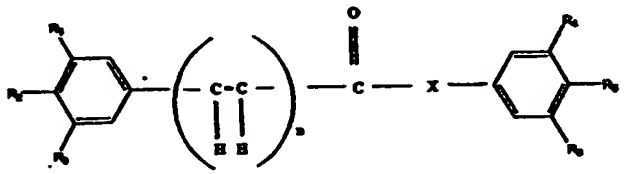
4. A process of treating baldness in a subject in need of such treatment comprising the step of administering to the subject an effective therapeutic amount of a compound of any of Tables 1-7.

5. A process of treating a skin disorder in a subject in need of such treatment comprising the step of administering to the subject an effective therapeutic amount of a compound of any of Tables 1-7.

6. A process of treating obesity in a subject in need of such treatment comprising the step of administering to the subject an effective therapeutic amount of a compound of any of Tables 1-7.

7. A process of treating benign prostatic hyperplasia or prostatitis in a subject in need of such treatment comprising the step of administering to the subject an effective therapeutic amount of a compound of any of Tables 1-7.

8. A compound of the formula:



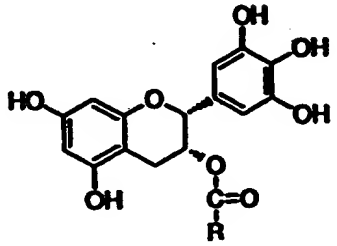
where x is $-NHCH_2CH_2-$ or $-CH=CH-$;

R_1, R_2 and R_3 each may be -H, -OH or $-OCH_3$, provided that only one of R_1, R_2, and R_3 may be -H;

R_4, R_5 and R_6 each may be -H, -OH, $-OCH_3$ or $-N(CH_3)_2$, provided that only one of R_4, R_5 and R_6 may be -H; and

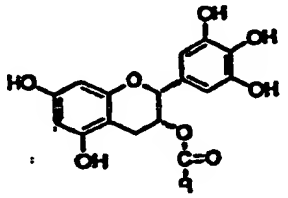
n is 0 or 1.

9. A compound of the formula:

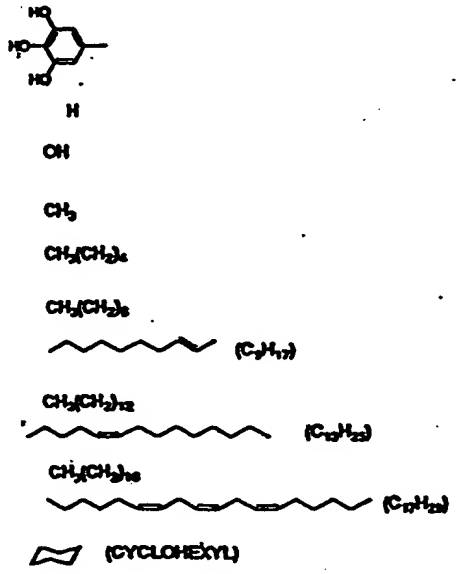


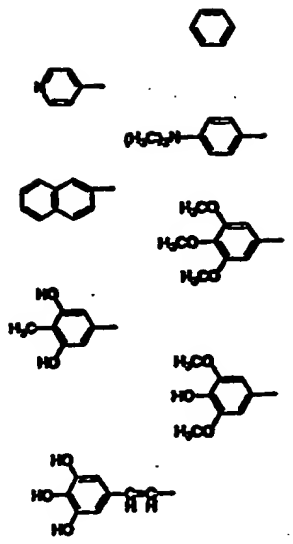
where R is a chain with 2 to 20 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with one to four double bonds and additional hydrogen.

10. A compound of the formula:



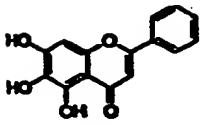
where R is



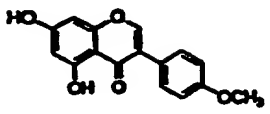


11. A method for the stabilization of compounds shown in Tables 1-7 comprising adding an effective ambient of an inorganic acid, an organic acid, or a natural product that contains these acids to maintain the acidity of the therapeutic preparations of these compounds at pH 3.0 to pH 6.8.

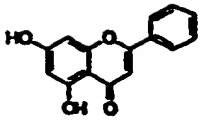
FIGURE 1 - FLAVANOIDS



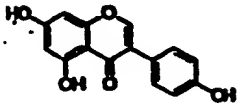
BAICALEIN



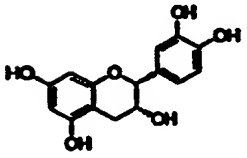
BIOCHANIN A



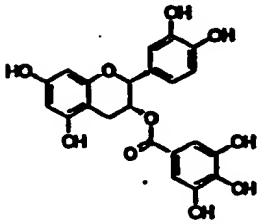
CHRYSIN



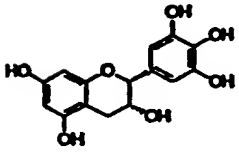
DAIDZEIN



EPICATECHIN



EPICATECHIN GALLATE



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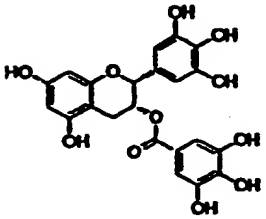

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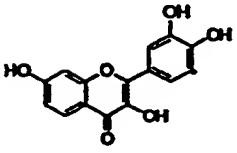

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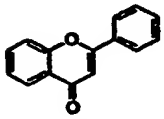
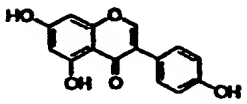
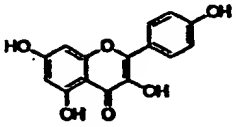


FLAVONE



GENISTEIN



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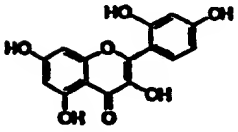

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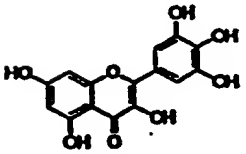

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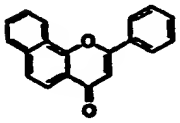
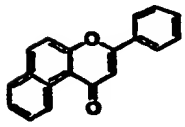
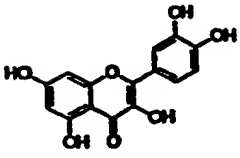


α-NAPTHOFLAVONE



β-NAPTHOFLAVONE



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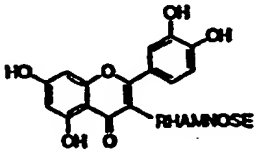

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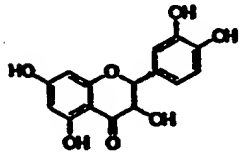
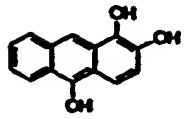


TAXIFOLIN

FIGURE 2 - CATECHOLS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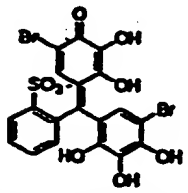
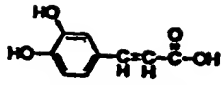
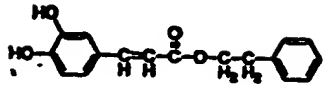
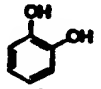
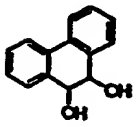
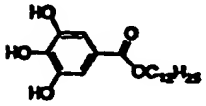

BROMOPYROGALLOL RED


CAFFEIC ACID


CAFFEIC ACID PHENETHYL ESTER


CATECHOL


CNC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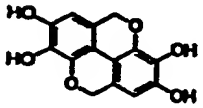
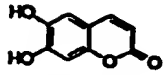

ELLAGIC ACID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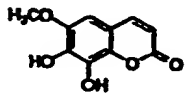
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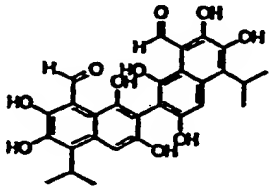
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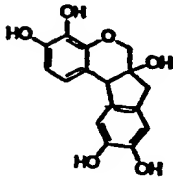
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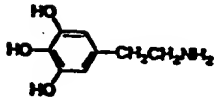
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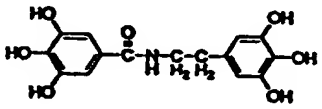
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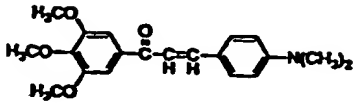
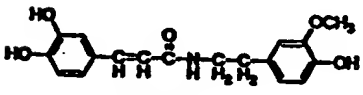

HZIV 82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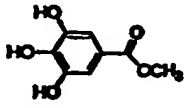
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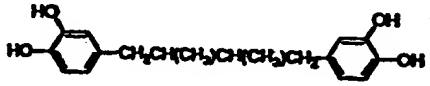
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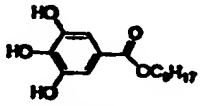
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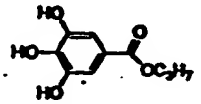
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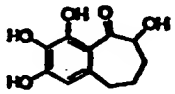
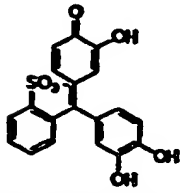

PURPUROGALLIN


PYROCATECHOL VIOLET


PYROGALLOL

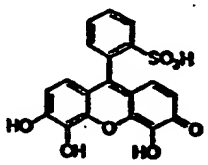
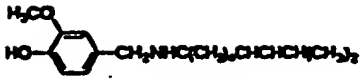

PYROGALLOL RED

FIGURE 3 - FERULIC ACID DERIVATIVES



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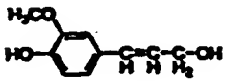
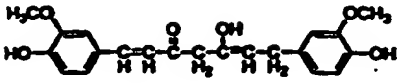
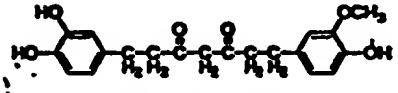


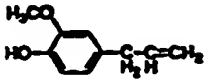
CONIFEROL



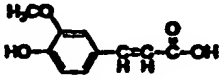
CURCUMIN



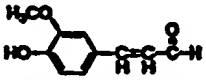
DEMETHOXYTETRAHYDROCURCUMIN



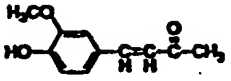
EUGENOL



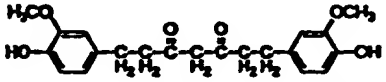
FERULIC ACID



4-HYDROXY-3-METHOXY-CINNAMALDEHYDE

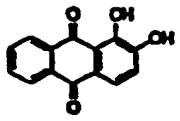


4-(4-HYDROXY-3-METHOXYPHENOL)-3-BUTEN-2-ONE

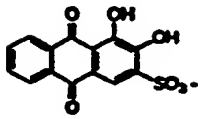


TETRAHYDROCURCUMIN
HZIV 81-2

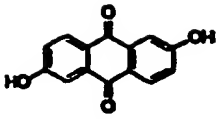
FIGURE 4 - QUINONES



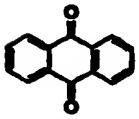
ALIZARIN



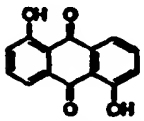
ALIZARIN RED S



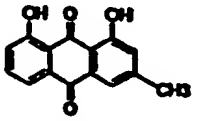
ANTHRAFLAVIC ACID



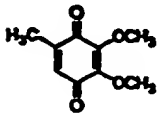
ANTHRAQUINONE



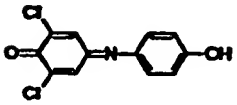
ANTHRARUFIN



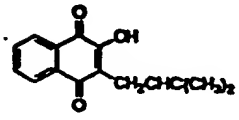
CHRYSOPHANIC ACID



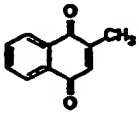
COENZYME Q1



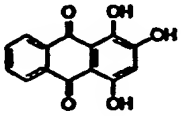
2,6-DICHLOROINDOPHENOL



LAPACHOL



MENADIONE



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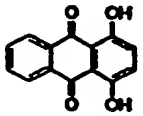
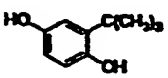
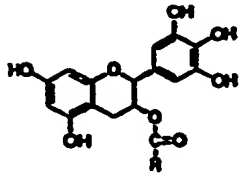


QUINIZARIN



t-BUTYLHYDROQUINONE

FIGURE 5 - EPIGALLOCATECHIN DERIVATIVES



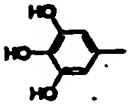
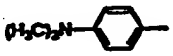
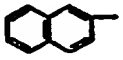
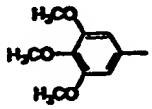
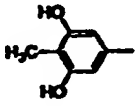
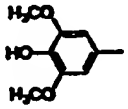
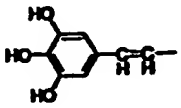
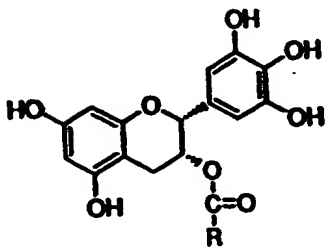
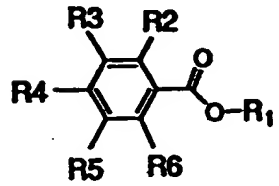
COMPOUND	R
EGCG	
EGC	H
HZIV 109	OH
HZIV 145	CH_3
HZIV 169	$CH_3(CH_2)_4$
HZIV 166	$CH_3(CH_2)_6$
HZIV 168	 (C_9H_{17})
HZIV 165	$CH_3(CH_2)_{12}$
HZIV 160	 $(C_{13}H_{25})$
HZIV 148	$CH_3(CH_2)_{16}$
HZIV 142	 $(C_{17}H_{29})$
HZIV 144	 (CYCLOHEXYL)
HZIV 74	
HZIV 107	
HZIV 92	
HZIV 120	
HZIV 63	
HZIV 68	
HZIV 75	
HZIV 134	

FIGURE 6 - EPICALLOCATECHIN DERIVATIVES



R: a chain with 2 to 20 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with one to four double bonds and additional hydrogen. These atoms can be in a straight chain or branched form, or in the form of aromatic ring structures, which may have a substitution of one to three carbon, alkyl, or halogenated alkyl, nitro, amino, methylated amino, carboxyl, or hydroxy groups, or halogen atoms.

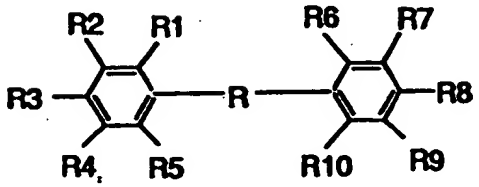
FIGURE 7 - GALLATES



R1: an alkyl chain with 2 to 20 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with one to four double bonds and additional hydrogen. These atoms can be in a straight chain or branched form, or in the form of aromatic ring structures, which may have substitution of one to three carbon alkyl or halogenated alkyl, nitro, amino, methylated amino, carboxyl, hydroxy groups or halogen atoms.

R2-5: an alkyl chain with 1 to 12 atoms from the group consisting of carbon, oxygen, sulfur, hydrogen and nitrogen, without or with hydroxy groups. These atoms can be in a straight chain or branched form, which may have substitution of one to three carbon alkyl or halogenated alkyl, nitro, amino, methylated amino, carboxyl groups and hydrogen or halogen atoms.

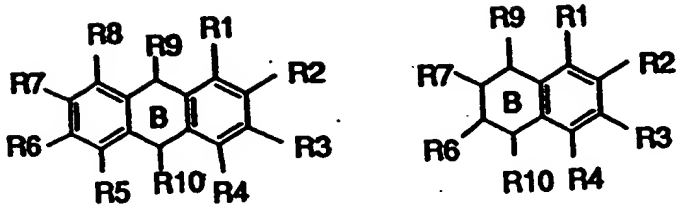
FIGURE 8 - CURCUMIN DERIVATIVES



R: An alkyl chain with 1 to 14 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with one to three double bonds, carbonyl, or hydroxyl groups and additional hydrogens. These atoms can be in a straight chain or branched form, or in the form of aromatic ring structures which may have substitution of one to three carbon alkyl or halogenated alkyl, nitro, amino, methylated amino, carboxyl, or halogen atoms.

R2-5: Hydroxy or methoxy groups or an alkyl chain with 1 to 10 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with hydroxy groups and additional hydrogens. These atoms can be in a straight chain or branched form, which may have substation of one to three carbon alkyl or halogenated alkyl, nitro, amino, methylated amino, carboxyl groups and hydrogen or halogen atoms.

FIGURE 9 - QUINONES AND CATECHOLS

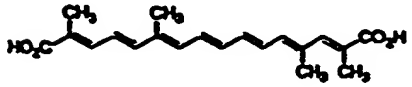


R1 - R8 can be 1 to 6 atoms that may consist of carbon, nitrogen, oxygen, and sulfur, and additional hydrogen or halogen atoms. They can be in the form of alkyl or halogenated alkyl, methoxy, nitro, hydroxy or amino groups. R9 and R10 can be hydroxy groups or in the form of quinones. Ring B can be in a saturated, aromatic or quinine structures.

FIGURE 10 - FATTY ACIDS

CONJUGATED OCTADECADIENOIC ACID: MIXTURE OF CIS AND TRANS 9,11 AND 10,12 OCTADECADIENOIC ACIDS (C18:2)

5,8,11,14-EICOSATETRAYNOIC ACID



CROCETIN

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/23041

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

cas online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,5,605,929 A (LIAO ET AL.) 25 February 1997, column 2 lines 21-32 and 47-56, figures 22 and 23, claims 1-8.	1-10
X	Wang, Z.Y. et al, "Interaction of epicathechins derived from green tea with rat hepatic cytochrome p-450" Chemical Abstracts 108:160935, 1988, see entire abstract.	1-7, 9, 10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
05 JANUARY 1999

Date of mailing of the international search report
03 FEB 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
AMELIA A. OWENS

Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/23041

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/35, 31/235, 31/165, 31/12, 31/075, 31/135, 31/24; C07D 311/04; C07C 49/105, 49/76

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

549/399; 564/184, 182; 568/306, 325, 331; 514/453, 455, 456, 532, 534, 544, 545, 617, 646, 649, 678, 679, 680, 681, 683, 690, 718, 720, 886, 909

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

549/399; 564/184, 182; 568/306, 325, 331; 514/453, 455, 456, 532, 534, 544, 545, 617, 646, 649, 678, 679, 680, 681, 683, 690, 718, 720, 886, 909

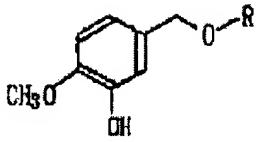
ISOVANILLYL ALCOHOL DERIVATIVE

Patent number: JP11209322
Publication date: 1999-08-03
Inventor: ONO TOSHIYA; YAMAGUCHI MASAKAZU; OBA TAKASHI; YAMAMURO AKIRA; FUJIKURA YOSHIAKI
Applicant: KAO CORP
Classification:
- international: C07C43/23; A61K7/00; A61K31/085
- european:
Application number: JP19980014351 19980127
Priority number(s):

Abstract of **JP11209322**

PROBLEM TO BE SOLVED: To obtain the subject new compound having a specific (branched) alkyl substituent, providing a reduced undesirable side effect and skin irritation, capable of rapidly imparting an effect of a strong warm feeling to skin when using the compound, capable of sustaining the feeling for a long period and useful as a skin preparation for external use.

SOLUTION: This new compound is the one of the formula [R is a 3-6C (branched) alkyl], e.g. isovanillyl n-propyl ether. The compound of the formula is obtained by reacting 3-hydroxy-4-methoxybenzyl halide with a corresponding alkoxide in a solvent or without the solvent. The compound is preferably used as a skin preparation for external use in a dosage form such as a lotion, an emulsion, a skin lotion, a dentifrice, a soap, a cataplasma and a cream regulated so as to include 0.001-10 wt.%, preferably 0.01-5 wt.% compound of the formula.



(19)日本国特許庁（ＪＰ）　(12)公開特許公報（A）　(11)特許出願公開番号

特開平11－209322

(43)公開日　平成11年(1999)８月３日

(51)Int.Cl.*	識別記号	FI	
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A61K 7/00		A61K 7/00	C
31/085	ADA	31/085	ADA

審査請求　未請求　請求項の数2　ＯＬ　（全５頁）

(21)出願番号　特願平10－14351

(22)出願日　平成10年(1998)１月27日

(71)出願人　000000918
花王株式会社
東京都中央区日本橋茅場町１丁目14番10号

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(72)発明者　大場　剛史
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(74)代理人　弁理士　有賀　三幸　（外４名）

最終頁に続く

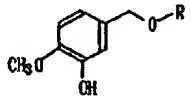
(54)【発明の名称】　イソバニリルアルコール誘導体

(57)【要約】

【課題】　安全で皮膚刺激感が低減され、使用時に温かい感覚を与える皮膚外用剤の提供。

【解決手段】　次の一般式（１）

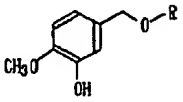
【化１】



（１）

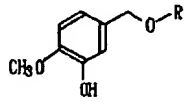
（式中、Ｒは炭素数３～６の直鎖または分岐鎖のアルキル基を示す。）で表わされるイソバニリルアルコール誘導体及びこれを有効成分とする皮膚外用剤。

【特許請求の範囲】
【請求項1】　次の一般式（1）
【化1】



（1）

（式中、Rは炭素数3～6の直鎖または分岐鎖のアルキル基を示す）で表わされるイソバニリルアルコール誘導体。
【請求項2】　請求項1記載のイソバニリルアルコール誘導体を有効成分とする皮膚外用剤。
【発明の詳細な説明】
【0001】
【発明の属する技術分野】本発明は新規なイソバニリルアルコール誘導体及びこれを有効成分とする、皮膚に塗布することにより温感を与え得る皮膚外用剤に関する。
【0002】
【従来の技術】従来より、使用時に温感を与える目的で多価アルコール、唐辛子末、唐辛子チンキ、唐辛子エキス、カプサイシン、ノナン酸バニルアミド、生姜溶液、メントール、カンファー、サリチル酸メチル等が皮膚外用剤に配合されている。またバニリルアルコール誘導体が皮膚刺激感覚に対して特殊な刺激を与え、これを溶媒で稀釈したものは温感を与えることが知られている（特開昭57－9729号公報）。さらにバニリルアルコール誘導体、水溶性界面活性剤及び水を配合することにより、皮膚に塗布した際、人体に好ましくない皮膚刺激感を低減し、かつ皮膚に速やかに温感を与えることができることが知られている（特開昭62－205007号公報）。
【0003】
【発明が解決しようとする課題】しかしながら、多価アルコール、唐辛子末等は、特異な刺激臭や強い皮膚刺激感を有していたり、温感効果が十分でない等の問題を有していた。また特開昭57－9729号公報の技術は、温感効果はあるが、同時に好ましくない皮膚刺激感も強く、この刺激感を低減するために使用量を減少させると、持続時間も短くなってしまうという問題を有していた。さらに特開昭62－205007号公報の技術は、皮膚刺激感を低下させることができるが、その効果は必ずしも満足できるものではなく、温感持続時間も短くなってしまうものであった。
【0004】従って、本発明は人体に好ましくない副作用や皮膚刺激感が低減され、使用時には速やかに温感を与えると共に適度の温感を長時間保持し得る化合物、及びかかる化合物を有効成分とする皮膚外用剤を提供することを目的とする。
【0005】
【課題を解決するための手段】かかる実情に鑑み、本発明者らは上記目的を達成するため鋭意研究したところ、意外にも特定のイソバニリルアルコール誘導体が、皮膚に対して強い温感効果を有すると共に、その効果が比較的長時間持続し、さらに人体に対して好ましくない皮膚刺激をほとんど生じないという従来の温感剤では実現しえない特性を有することを見出し、本発明を完成させた。
【0006】すなわち本発明は、次の一般式（1）
【0007】
【化2】



（1）

【0008】（式中Rは炭素数3～6の直鎖または分岐鎖のアルキル基を示す。）で表わされるイソバニリルアルコール誘導体、及びかかるイソバニリルアルコール誘導体を有効成分とする皮膚外用剤を提供するものである。
【0009】
【発明の実施の形態】本発明のイソバニリルアルコール誘導体としては、一般式（1）中Rがn－プロピル基、i－プロピル基、n－ブチル基、i－ブチル基、n－ペンチル基であることが好ましい。
【0010】本発明のイソバニリルアルコール誘導体は、公知の種々の方法により合成することができる。例えばウィリアムソンのエーテル合成法により、3－ヒドロキシ－4－メトキシベンジルハライドとアルコキシドとを、またはイソバニリルアルコキシドとアルキルハライドとを、無溶媒または溶媒中で反応させて合成することができる。
【0011】あるいは例えば次の方法で合成することもできる。すなわち、対応するアルコールに濃塩酸や濃硫酸等の強酸を混合し、これにイソバニリルアルコールを加えて反応させ、目的物を得ることができる。ここで強酸はアルキルアルコールの0．1～30モル%程度添加することが好ましい。また加熱温度は20～90℃程度であることが好ましい。さらにアルキルアルコールはイソバニリルアルコールに対してモル比で2～20倍程度用いることが好ましい。
【0012】本発明の皮膚外用剤は、上記のイソバニリルアルコール誘導体を有効成分として含有するもので、上記イソバニリルアルコール誘導体を1種または2種以上混合して用いることができる。あるいは、唐辛子末、多価アルコール等の他の温感を与える化合物を併用することにより、皮膚刺激感を高めることなく温感効果をさらに向上させることもできる。本発明の皮膚外用剤の形態としては特に制限はなく、例えばローション、乳液、化粧水、歯磨剤、液体石ケン、固体石ケン、クリーム状ヘアーコンディショナー、パップ剤、クリーム、ボディ

ーシャンプー、ハンドクリーム、ジェル、軟膏等を挙げることができる。かかる製剤とするために、必要に応じて賦形剤、増量剤、結合剤、湿潤化剤、崩壊剤、油性物質、界面活性剤、滑沢剤、分散剤、緩衝剤、保存剤、防腐剤、着色剤、香料等を適宜配合することができる。

【００１３】本発明のイソバニリルアルコール誘導体の皮膚外用剤中の含有量は、０．００１～１０重量％であることが好ましく、０．０１～５重量％であることが特に好ましい。０．００１～１０重量％であれば、皮膚に温感を与える効果が大きく、さらに好ましくない皮膚刺激感を与えることが極めて少ない。

【００１４】本発明の皮膚外用剤は、例えばイソバニリルアルコール誘導体及びその他の添加剤を混合し、適宜撹拌等することにより得ることができる。

【００１５】

【実施例】次に実施例を示して本発明をさらに詳細に説明するが、本発明は以下の実施例に限定されるものではない。

【００１６】実施例１　　化合物（１）イソバニリルｎ－プロピルエーテルの合成

磁器撹拌器、還流冷却管を備えた１００mL容の２口フラスコにｎ－プロピルアルコール２３．４ｇ（３８９mmol）及び濃塩酸０．１８mLを加え、７０℃に加熱し撹拌した。これにイソバニリルアルコール６．００ｇ（３８．９mmol）を加え、３０分間反応させた。反応終了後室温まで冷却し、飽和炭酸水素ナトリウム溶液を加えて塩酸を中和した。次いで酢酸エチルで抽出し、抽出した油層を飽和食塩水で十分洗浄し、無水硫酸マグネシウムで乾燥した後、溶媒を留去して黄色油状物を得た。この黄色油状物を減圧蒸留（１５０～１６０℃／０．１mmHg）によって精製し、標記化合物イソバニリルｎ－プロピルエーテル（１－１）３．２８ｇ（イソバニリルアルコールに対して４３．０％）を得た。得られた化合物（１）の物性は次の通りである。

【００１７】^{1}H-NMR($CDCl_3$, δ)：0.93(t,3H,J=7.4Hz), 1.61(m,2H),3.40(t,2H,J=6.7Hz), 3.88(s,3H), 4.40(s,2H), 5.63(s,1H),6.82-6.93(m,3H)

IR(cm^{-1})：3436, 2968, 2940, 2864, 1596, 1514, 1458, 1446, 1370, 1278,1154, 1128, 1092, 1028, 804, 760

【００１８】実施例２　　化合物（２）イソバニリルｉｓｏ－プロピルエーテルの合成

上記実施例１で用いたｎ－プロピルアルコールをｉｓｏ－プロピルアルコール１９．５ｇ（３２４mmol）に代えて、イソバニリルアルコール４．７５ｇ（３０．８mmol）を用いて上記実施例１と同様の方法で行い、減圧蒸留（１６０℃／０．１mmHg）精製によって標記化合物イソバニリルｉｓｏ－プロピルエーテル（２）２．５６ｇ（イソバニリルアルコールに対して４２．３％）を得た。得られた化合物（２）の物性は次の通りである。

【００１９】^{1}H-NMR($CDCl_3$, δ)：1.20(d,6H,J=6.0Hz), 3.66(sept,1H,J=6.0Hz),3.88(s,3H), 4.41(s,2H), 5.61(s,1H), 6.82-6.94(m,3H)

IR(cm^{-1})：3448, 2976, 2940, 2876, 1596, 1516, 1446, 1382, 1278, 1126,1030, 800, 760

【００２０】実施例３　　化合物（３）イソバニリルｎ－ブチルエーテルの合成

上記実施例１で用いたｎ－プロピルアルコールをｎ－ブチルアルコール８２．０ｇ（１．１１mol）に代えて、イソバニリルアルコール１７．０ｇ（１１０．３mmol）、濃塩酸０．４８ｇを用いて上記実施例１と同様の方法で行い、減圧蒸留（１７０℃／０．２mmHg）精製によって標記化合物イソバニリルｎ－ブチルエーテル（３）１２．１９ｇ（イソバニリルアルコールに対して５２．６％）を得た。得られた化合物（３）の物性は次の通りである。

【００２１】^{1}H-NMR($CDCl_3$, δ)：0.91(t,3H,J=7.2Hz), 1.36-1.62(m,4H),3.44(t,2H,J=8.0Hz), 3.88(s,3H), 4.40(s,2H), 5.62(s,1H),6.62-6.93(m,3H)

IR(cm^{-1})：3448, 2964, 2940, 2872, 1596, 1514, 1466, 1374, 1278, 1128,1094, 1030, 802, 760

【００２２】実施例４　　化合物（４）イソバニリルｉｓｏ－ブチルエーテルの合成

上記実施例１で用いたｎ－プロピルアルコールをｉｓｏ－ブチルアルコール２８．８ｇ（３７９mmol）に代え、減圧蒸留を１６０～１７０℃／０．１mmHgで行った以外は上記実施例１と同様の方法で行い、標記化合物イソバニリルｉｓｏ－ブチルエーテル（４）４．２３ｇ（イソバニリルアルコールに対して５１．７％）を得た。得られた化合物（４）の物性は次の通りである。

【００２３】^{1}H-NMR($CDCl_3$, δ)：0.91(d,6H,J=6.6Hz), 1.89(sept,1H,J=6.6Hz),3.20(d,2H,J=6.6Hz), 3.88(s,3H), 4.41(s,2H), 5.62(s,1H),6.82-6.93(m,3H)

IR(cm^{-1})：3248, 3036, 3004, 2956, 2876, 1592, 1514, 1460, 1448, 1370,1280, 1132, 1066, 1026, 756, 640

【００２４】実施例５　　化合物（５）イソバニリルアミルエーテルの合成

上記実施例１で用いたｎ－プロピルアルコールをｎ－アミルアルコール３４．３ｇ（３８９mmol）に代え、減圧蒸留を１６０～１７０℃／０．１mmHgで行った以外は上記実施例１と同様の方法で行い、標記化合物イソバニリルｎ－アミルエーテル（５）４．３８ｇ（イソバニリルアルコールに対して５０．２％）を得た。得られた化合物（５）の物性は次の通りである。

【００２５】^{1}H-NMR($CDCl_3$, δ)：0.89(t,3H,J=6.9Hz), 1.33(m,4H), 1.56-1.63(m,2H),3.43(t,2H,J=6.6Hz), 3.88(s,3H), 4.40(s,2H), 5.61(s,1H),6.82-6.93(m,3H)

IR(cm^{-1})：3448, 2940, 2864, 1596, 1514, 1460, 1368, 1276, 1128, 1094,1028, 802, 760

【００２６】実施例６　化合物（６）イソバニリルｉｓｏ－アミルエーテルの合成
上記実施例１で用いたｎ－プロピルアルコールをｉｓｏ－アミルアルコール２８．６ｇ（３２４ｍｍｏｌ）に代え、減圧蒸留を１６０～１７０℃／０．１ｍｍＨｇで行った以外は上記合成例１と同様の方法で行い、標記化合物イソバニリルｉｓｏ－アミルエーテル（６）３．２５ｇ（イソバニリルアルコールに対して４４．７％）を得た。得られた化合物（６）の物性は次の通りである。
【００２７】^{1}H-NMR($CDCl_3$, δ)：0.89(d,6H,J=6.5Hz), 1.50(q,2H,J=6.8Hz),1.73(m,1H), 3.46(t,2H,J=6.8Hz), 3.88(s,3H), 4.40(s,2H), 5.60(s,1H),6.82-6.93(m,3H)
IR(cm^{-1})：3432, 2960, 2872, 1596, 1514, 1464, 1446, 1372, 1278, 1128,1092, 1028, 802, 760
【００２８】実施例７　化合物（７）イソバニリルｎ－ヘキシルエーテルの合成
上記実施例１で用いたｎ－プロピルアルコールをｎ－ヘキシルアルコール３３．０ｇ（３２４ｍｍｏｌ）に代え、減圧蒸留を１６０～１７０℃／０．１ｍｍＨｇで行った以外は上記実施例１と同様の方法で行い、標記化合物イソバニリルｎ－ヘキシルエーテル（７）４．８３ｇ（イソバニリルアルコールに対して６２．６％）を得た。得られた化合物（７）の物性は次の通りである。
【００２９】^{1}H-NMR($CDCl_3$, δ)：0.88(t,3H,J=6.8Hz), 1.29-1.63(m,8H),3.43(t,2H,J=6.6Hz), 3.88(s,3H), 4.40(s,2H), 5.60(s,1H),6.82-6.93(m,3H)
IR(cm^{-1})：3440, 2936, 2864, 1596, 1514, 1462, 1446, 1372, 1278, 1128,1096, 1030, 802, 758
【００３０】試験例１
表１に示す配合で試験溶液を作成した。
【００３１】
【表１】

	試験溶液					
	(1)	(2)	(3)	(4)	(5)	(6)
化合物（１）	0.05	－	－	－	－	－
化合物（２）	－	0.05	－	－	－	－
化合物（３）	－	－	0.05	－	－	－
化合物（４）	－	－	－	0.05	－	－
化合物（５）	－	－	－	－	0.05	－
カプサイシン	－	－	－	－	－	0.005
５０％エタノール水	バランス	バランス	バランス	バランス	バランス	バランス

【００３２】１０人の健常男性の前腕内側に、上記で得られた各試験溶液を１０μＬ塗布し、塗布直後、３０分後、及び１時間後の温感と皮膚刺激感について調査した。温感または皮膚刺激感をわずかでも感じられると答えた人の数を表２に示す。
【００３３】
【表２】

	塗布直後		３０分後		１時間後	
	温感	皮膚刺激感	温感	皮膚刺激感	温感	皮膚刺激感
試験溶液（１）	8	6	6	4	5	1
試験溶液（２）	6	6	4	3	4	1
試験溶液（３）	10	6	8	4	6	2
試験溶液（４）	7	7	4	2	3	1
試験溶液（５）	6	5	4	2	3	0
試験溶液（６）	10	10	6	9	2	8

【００３４】表２より、試験溶液（１）～（５）は、十分な温感が比較的長時間持続し、かつ皮膚刺激感が弱いという優れた効果を有することが確認された。またかかる効果は従来の温感剤からは得られないものであることが確認された。
【００３５】実施例８
表３に示す配合割合で常法により化粧水を製造した。
【００３６】
【表３】

成分	重量％
グリセリン	１５．０
ポリエチレングリコール（PEG1500）	２．０

ヒアルロン酸	0．05
ジプロピレングリコール	5．0
化合物（1）	0．05
精製水	バランス

【0037】

【発明の効果】本発明の皮膚外用剤は、本発明のイソバニリルアルコール誘導体を有効成分として含有することにより、人体に好ましくない副作用が皮膚刺激感が低減され、使用時には速やかに肌に温感を与えると共に、適度の温感を長時間保持することができる。

フロントページの続き

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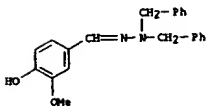
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9940062	A1	19990812	WO 1999-DK53	19990203
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9926102	A1	19990823	AU 1999-26102	19990203

PRIORITY APPLN. INFO.: DK 1998-159 A 19980205
US 1998-74001P P 19980209
WO 1999-DK53 W 19990203
OTHER SOURCE(S): MARPAT 131:157643
AB Hydrazone derivs. of formula R1R2C=NNR3R4 (I) or R1R2C=NN=CR3R4 (II) [R1-R4 = H, C1-8 alkyl, C3-8 cycloalkyl, OH, acyl, C1-6 alkoxy, NO2, cyano, (un)substituted carbonyl, (un)substituted amino, (un)substituted sulfonamide, (un)substituted (hetero)aryl, etc.; R1 and R2 or R3 and R4 may together form an (un)subtituted hetero- or carbocycle], were prepd for use as medicaments in therapy esp. in the treatment of diseases related to glucose metabolic pathways. Specifically, claimed compds. I and II were prepd. for use in the treatment or prevention of diseases of the endocrinol. system, preferably hyperglycemia, NIDDM, or diabetes , and for treatment of glycogen storage disease or hypoglycemia. Compns. of the invention are claimed to exhibit glucose-6-phosphatase inhibitory activity with IC50 values of less than 100 .mu.M (no data). Thus, 4-chlorobenzaldehyde was added to N,N-dibenzylhydrazine in DMF followed by addn. of tri-Et orthoformate to form N,N-dibenzyl-N'-(4-chlorobenzylidene)hydrazine, i.e., I (where R1 = H, R2 = C6H4-p-Cl, and R3 = R4 = CH2Ph), in 94% yield.
IT 237402-36-7P, 4-(Dibenzylhydrazonomethyl)-2-methoxyphenol
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(prepn. of hydrazone derivs. for treatment or prevention of diseases of the endocrinol. system, preferably hyperglycemia, NIDDM, or diabetes, and for treatment of glycogen storage disease or hypoglycemia)
RN 237402-36-7 CAPLUS
CN Benzaldehyde, 4-hydroxy-3-methoxy-, bis(phenylmethyl)hydrazone (9CI) (CA INDEX NAME)

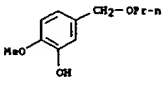


REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:481288 CAPLUS
DOCUMENT NUMBER: 131:116071
TITLE: Preparation of ethers of isovanillyl alcohols as skin treatment agents for external use
INVENTOR(S): Ono, Toshiya; Yamaguchi, Masashi; Ohba, Takeshi; Yamamuro, Akira; Fujikura, Yoshiaki
PATENT ASSIGNEE(S): Kao Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 5 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 11209322	A2	19990803	JP 1998-14351	19980127

PRIORITY APPLN. INFO.: JP 1998-14351 19980127
OTHER SOURCE(S): MARPAT 131:116071
AB Title compds. I (R = C3-6 linear or branched alkyl), which give a feeling of warmth and reduced stimulation, are prepd. Isovanillyl alc. was etherified with Pr alc. in the presence of HCl at 70.degree. for 30 min to give 43.0% isovanillyl Pr ether, which give feeling of warmth on the skin.
IT 233255-79-3P 233255-80-6P 233255-81-7P
233255-82-8P 233255-83-9P 233255-84-0P
233255-85-1P
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(prepn. of ethers of isovanillyl alcs. as skin treatment agents for external use)
RN 233255-79-3 CAPLUS
CN Phenol, 2-methoxy-5-(propoxymethyl)- (9CI) (CA INDEX NAME)



RN 233255-80-6 CAPLUS
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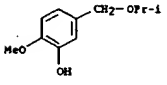
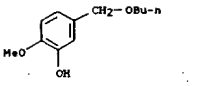
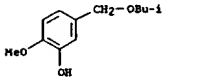


RN 233255-81-7 CAPLUS
CN Phenol, 5-(butoxymethyl)-2-methoxy- (9CI) (CA INDEX NAME)

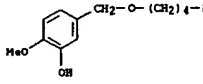
L7 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2003 ACS (Continued)



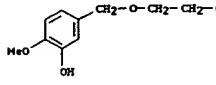
RN 233255-82-8 CAPLUS
CN Phenol, 2-methoxy-5-[(2-methylpropoxy)methyl]- (9CI) (CA INDEX NAME)



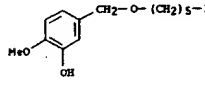
RN 233255-83-9 CAPLUS
CN Phenol, 2-methoxy-5-[(pentyloxy)methyl]- (9CI) (CA INDEX NAME)



RN 233255-84-0 CAPLUS
CN Phenol, 2-methoxy-5-[(3-methylbutoxy)methyl]- (9CI) (CA INDEX NAME)



RN 233255-85-1 CAPLUS
CN Phenol, 5-[(hexyloxy)methyl]-2-methoxy- (9CI) (CA INDEX NAME)



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08/997,416 24 December 1997 (24.12.97) US

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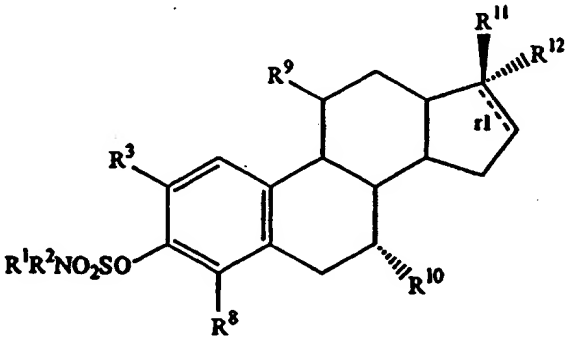
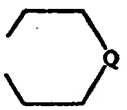
(54) Title: ESTRONE SULFAMATE INHIBITORS OF ESTRONE SULFATASE, AND ASSOCIATED PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE



(I)



(II)

(57) Abstract

Novel compounds useful as inhibitors of estrone sulfatase are provided. The compounds have structural formula (I) wherein r1 is an optional double bond, R^1 and R^2 are selected from the group consisting of hydrogen and lower alkyl, or together form a cyclic substituent (II) wherein Q is NH, O or CH_2, and the other various substituents are as defined herein. Pharmaceutical compositions and methods for using the compounds of formula (I) to treat estrogen-dependent disorders are provided as well.

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ESTRONE SULFAMATE INHIBITORS OF ESTRONE SULFATASE, AND ASSOCIATED PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE

Technical Field

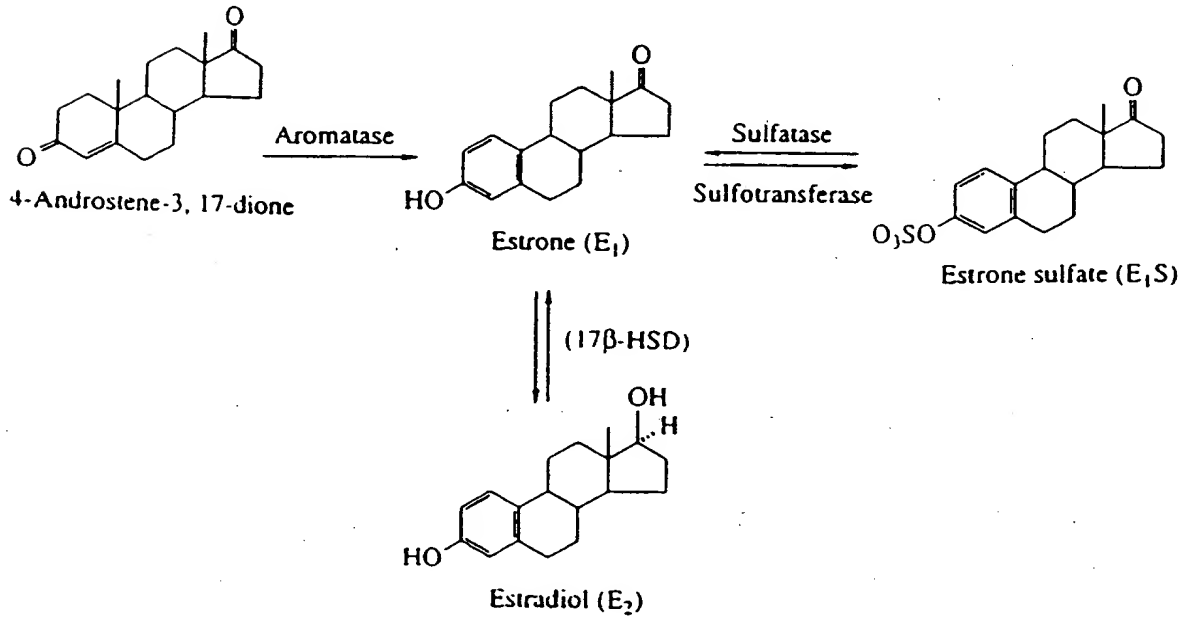
The present invention relates generally to steroid hormones, and more specifically relates to novel steroids which are inhibitors of the enzyme estrone sulfatase. The invention additionally relates to methods for inhibiting estrone sulfatase activity, to treatment of disorders that are estrogen-dependent, i.e., are estrogen-induced or estrogen-stimulated, and to pharmaceutical compositions containing one or more of the novel compounds.

Background

Breast cancer is one of the most prevalent types of cancer, and epidemiological and clinical studies have shown that approximately one-third of breast tumors are estrogen-dependent. This means that estrogens are required for the growth of such breast tumors in both premenopausal and postmenopausal patients. In postmenopausal women, in whom breast cancer most commonly occurs, breast tumor concentrations of estrone and estradiol are considerably higher than blood estrogen levels. Although retention of estrogens in breast tumors by high-affinity binding proteins contributes to the level of estrogens in tumors, estrogen concentrations in the breast are higher than plasma levels in breast cancer patients regardless of whether their tumors are estrogen receptor-positive (ER+) or receptor-negative (ER-). *In situ* formation of estrogen from estrogen biosynthetic precursors within tumors is now known to make a major contribution to the estrogen content of breast tumors.

The principal naturally occurring estrogens are 17β-estradiol, estrone, and estriol. The enzymes required for estradiol biosynthesis (i.e., aromatase, 17β-hydroxy-steroid dehydrogenase, and estrone sulfatase) are present in normal and malignant breast tissues. Blood concentrations of estrone sulfate are 8- to 10-fold

greater than those of unconjugated free estrone, and breast tissue concentrations of estrone sulfatase activity, the enzyme responsible for the conversion of estrone sulfate to estrone, are a thousand-fold higher than those of aromatase activity. Together, these findings suggest that estrone sulfatase plays a key role in regulating the formation of estrogens within breast tumors, particularly in postmenopausal women. *See, e.g.:* Thijsen et al., *Ann. N.Y. Acad. Sci.* 464:106-116 (1986); Santner et al., *J. Clin. Endocrinol. Metabol.* 59(1):29-33 (1984); Evans et al., *J. Steroid Biochem. Mol. Biol.* 39:493-499 (1991); Pasqualini et al., *J. Steroid Biochem. Mol. Biol.* 41(308):323-329 (1992); Vignon et al., *Endocrinology* 106(4):1079-1086 (1980); and Santner et al., *Int. J. Cancer* 54:119-124 (1993).



Biosynthesis of estrogens

There is additional evidence of the relative significance of the aromatase and estrone sulfatase pathways in providing sufficient estrogen to sustain tumor growth. In postmenopausal women, the levels of estradiol in breast tumor tissues are 10 to 40 times higher than in plasma and are similar to those in premenopausal women, even though plasma estrogen levels are much lower after the menopause. This concentration gradient is not entirely due to estradiol uptake and binding to estrogen receptors, since tissue estradiol levels correlate poorly with estrogen receptor levels.

In situ production of estradiol, through either the aromatase or the estrone sulfatase pathway, could affect this gradient. The level of estrone sulfate present in the serum of postmenopausal women is 10 times higher than the level of free estrogens (Prost et al., *Cancer Res.* 44:661-664 (1984)). Serum estrone sulfate levels are also higher in postmenopausal women with breast cancer than in normal postmenopausal women (Purohit et al., *Int. J. Cancer* 50:901-905 (1992)). Also, sulfatase levels in tumors are much higher than aromatase levels (Pasqualini et al., *J. Steroid Biochem.* 34(1-6):155-163 (1989); Adams et al., *Cancer Res.* 39:5124-5126 (1979)). The absolute levels of aromatase activity in tumors are relatively low, ranging from 5 to 80 pmol/g protein/h. Bradlow (Bradlow et al., *Cancer Res.* (Suppl.) 42:3382s-3386s (1982)) and others consider this degree of tumor aromatase activity too low for a biologically meaningful level of estradiol to be synthesized locally within the tumor.

Quantitative information on the local production of estrogen shows that the sulfatase activity in breast tumors is more than 10 times the aromatase activity. When sulfatase and aromatase activity in human tumors were compared at physiological levels of substrate, sulfatase produced 2.8 pmol estrone/g protein/h while aromatase produced only 0.27 pmol/g protein/h. Consequently, estrone sulfate represents one of the most important precursors for tissue production of estradiol, and estrone sulfatase is a quantitatively more important local route for estrogen production than aromatase.

To date, little work has been done in the development of estrone sulfatase inhibitors. Li et al., *Steroids* 60:299-306 (1995), evaluate several compounds as potential inhibitors of human placental sterylsulfatase, but do not identify any highly

potent estrone sulfatase inhibitors. Similarly, Duncan et al., *Cancer Research* 53:298-303 (1993), evaluate a potential estrone sulfatase inhibitor, estrone-3-methylthiophosphonate, but conclude that the experimental work done with that compound would hopefully lead to development of "more efficient" inhibitors of the enzyme.

Accordingly, the present invention is directed to novel compounds that are extremely effective estrone sulfatase inhibitors. The invention thus represents a significant advance in the art, particularly in the treatment of breast cancer and other diseases and conditions that are potentiated by the presence of estrogens.

In addition to the references cited above, the following pertain to one or more aspects of the invention and as much may be of background interest to those skilled in the art: Howarth et al., *J. Med. Chem.* 37:219-221 (1994) and PCT Publication No. WO93/05064 relate to certain estrone sulfamates as inhibitors of steroid sulfatases, with Howarth et al. specifically focused on inhibition of estrone sulfatase. In addition, Dibbelt et al., *J. Steroid Biochem. & Molec. Biol.* 50(5/6):261-266 (1994) evaluates estrone sulfamate as a potential inhibitor of human placental sterylsulfatase, while Li et al., *Steroids* 58:106-111 (1993), and Purohit et al., *Biochemistry* 34:11508-11514 (1995) also, discuss estrone sulfamate as a potential enzyme inhibitor. However, the compounds described in these and other references are believed to give rise to estrogenic products upon hydrolysis, unlike the novel compounds provided herein.

Summary of the Invention

Accordingly, it is a primary object of the invention to address the above-mentioned need in the art by providing novel compounds useful as inhibitors of estrone sulfatase.

It is another object of the invention to provide novel estrone sulfatase inhibitors which are non-estrogenic.

It is still another object of the invention to provide such compounds which do not give rise to estrogenic products when hydrolyzed.

It is yet another object of the invention to provide novel estrone sulfatase inhibitors which are anti-estrogenic.

It is an additional object of the invention to provide such compounds which give rise to anti-estrogenic products upon hydrolysis.

It is another object of the invention to provide a method for inhibiting estrone sulfatase activity using the novel compounds.

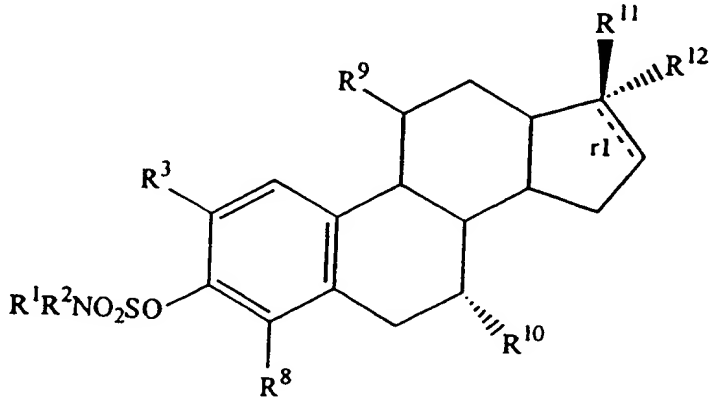
It is a further object of the invention to provide a method for treating an individual with a disorder that is estrogen-dependent, i.e., an estrogen-induced or estrogen-stimulated condition or disease, by administering to the individual an effective estrone sulfatase inhibiting amount of a novel compound as provided herein, or a pharmaceutically acceptable salt thereof.

It is yet a further object of the invention to provide a pharmaceutical composition for treating an individual with a disorder that is estrogen-dependent, the composition comprising a pharmaceutically acceptable carrier and an effective estrone sulfatase inhibiting amount of a novel compound as provided herein or a pharmaceutically acceptable salt thereof.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

In one embodiment, the invention relates to novel compounds having the structure of Formula (I)

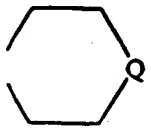
(I)



wherein:

r1 is an optional double bond;

R^1 and R^2 are selected from the group consisting of hydrogen and lower alkyl, or together form a cyclic substituent (II)

(II) 

wherein Q is NH, O or CH_2;

R^3 is selected from the group consisting of hydrogen, -CN, $-NO_2$, $-COOR^4$ wherein R^4 is hydrogen or lower alkyl, $-(CH_2)_nOR^5$ and $-(CH_2)_nNR^6R^7$ wherein n is an integer in the range of 0 to 6, R^5 is hydrogen or lower alkyl, and R^6 and R^7 are selected from the group consisting of hydrogen, lower alkyl and lower acyl, or together form the cyclic substituent (II);

R^8 is selected from the group consisting of hydrogen, $-NO_2$, and NR^6R^7;

R^9 and R^{10} are independently selected from the group consisting of hydrogen and lower alkyl; and

one of R^{11} and R^{12} is hydrogen and the other is lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively, or R^{11} and R^{12} together form =O or $=CR^{13}R^{14}$ in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, -CN, $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ and $-COOR^4$.

The invention also relates to pharmaceutical compositions containing one or more compounds of structural formula (I), and further relates to methods of using the compounds to inhibit estrone sulfatase activity and to treat individuals with disorders that are estrogen-dependent.

Detailed Description of the Invention

Definitions and Nomenclature:

Before the present compounds, compositions and methods are disclosed and described, it is to be understood that this invention is not limited to specific reagents or reaction conditions, specific pharmaceutical carriers, or particular administration regimens, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an estrone sulfatase inhibitor" includes mixtures of estrone sulfatase inhibitors, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

The term "alkyl" as used herein refers to a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like, as well as cycloalkyl groups such as cyclopentyl, cyclohexyl and the like. Preferred alkyl groups herein contain 1 to 12 carbon atoms. The term "lower alkyl" intends an alkyl group of one to six carbon atoms, preferably one to four carbon atoms.

The term "alkenyl" as used herein refers to a branched or unbranched hydrocarbon group of 2 to 24 carbon atoms containing at least one double bond, such as ethenyl, *n*-propenyl, isopropenyl, *n*-butenyl, isobutenyl, octenyl, decenyl, tetradecenyl, hexadecenyl, eicosenyl, tetracosenyl and the like. Preferred alkenyl groups herein contain 2 to 12 carbon atoms. The term "lower alkenyl" intends an alkenyl group of two to six carbon atoms, preferably two to four carbon atoms. The term "cycloalkenyl" intends a cyclic alkenyl group of three to eight, preferably five or six, carbon atoms.

The term "alkynyl" as used herein refers to a branched or unbranched hydrocarbon group of 2 to 24 carbon atoms containing at least one triple bond, such as ethynyl, *n*-propynyl, isopropynyl, *n*-butynyl, isobutynyl, octynyl, decynyl and the like. Preferred alkynyl groups herein contain 2 to 12 carbon atoms. The term "lower alkynyl" intends an alkynyl group of two to six carbon atoms, preferably two to four carbon atoms.

The term "alkoxy" as used herein intends an alkyl group bound through a single, terminal ether linkage; that is, an "alkoxy" group may be defined as -OR where R is alkyl as defined above. A "lower alkoxy" group intends an alkoxy group containing one to six, more preferably one to four, carbon atoms.

The term "acyl" is used in its conventional sense to refer to a molecular substituent RCO- where R is alkyl as defined above. The term "lower acyl" refers to an acyl group wherein R contains one to six, more preferably one to four, carbon atoms.

The term "ester" is used herein in its conventional sense to refer to a molecular substituent R(CO)O- where R is alkyl as defined above. A "lower alkyl ester" group intends an ester group containing one to six, more preferably one to four, carbon atoms, i.e., a lower acyloxy group.

The term "aryl" as used herein refers to a monocyclic aromatic species of 5 to 7 carbon atoms, and is typically phenyl. Optionally, these groups are substituted with one to four, more preferably one to two, lower alkyl, lower alkoxy or hydroxyl substituents.

The term "sulfamate" is used in its conventional sense to refer to a molecular substituent having the general formula $-O(SO_2)NR^1R^2$. In some instances, a sulfamate group may be drawn as $R^1R^2NO_2SO-$, but it is to be understood that the sulfur atom is directly bonded to each of the three oxygen atoms as well as to the nitrogen atom, with no oxygen atoms separatingR^1 and R^2 may be H; optionally, they may represent other substituents as discussed elsewhere herein.

The term "inhibitor" as used herein is intended to include both reversible enzyme inhibitors and irreversible enzyme inhibitors, i.e., enzyme inactivators.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, the phrase "optional double bond" used to refer to the dotted line in the structure of formula (I) indicated as "r1" means that either a single bond or a double bond is present.

By the terms "effective amount" or "pharmaceutically effective amount" or "estrone sulfatase inhibiting amount" of an agent as provided herein are meant a nontoxic but sufficient amount of the agent to provide the desired level of enzyme inhibition and corresponding therapeutic effect. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, and the particular enzyme inhibitor and mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

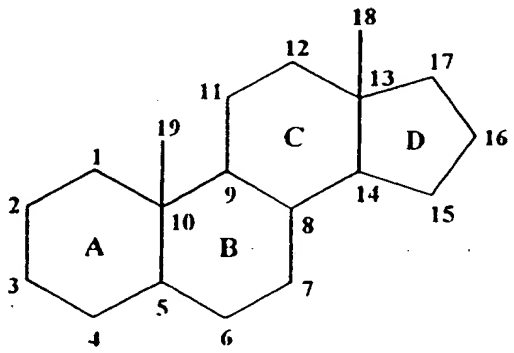
By "pharmaceutically acceptable carrier" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected estrone sulfatase inhibitor without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. Similarly, a "pharmaceutically acceptable" salt or a "pharmaceutically acceptable" ester of a novel compound as provided herein is a salt or ester which is not biologically or otherwise undesirable.

The term "estrogenic" relates to the ability to produce some or all of the effects produced by estrogens. Conversely, the term "non-estrogenic" is used to refer to compounds which do not produce estrogenic effects. The preferred compounds described herein are non-estrogenic, and upon hydrolysis do not give rise to estrogenic products. The term "reduced estrogenic activity" refers to a compound that has 60 % or less of the estrogenic activity of estradiol. A compound may also be referred to herein as having "substantially no estrogenic activity,"

meaning that the compound has less than about 5%, preferably less than about 2%, of the estrogenic activity of estradiol.

The term "anti-estrogenic" is used herein to mean the ability to inhibit or modify the effects produced by estrogens. An "anti-estrogenic" compound tends to inhibit the activity or the *in situ* production of estrogens such as estradiol, following administration to a mammalian individual. Preferred compounds of the invention are anti-estrogenic in nature and give rise to anti-estrogenic products upon hydrolysis. A "pure" anti-estrogenic compound as used herein refers to an anti-estrogenic compound which has no estrogenic activity, or substantially no estrogenic activity.

In describing the location of groups and substituents, the following numbering systems will be employed. This system is intended to conform the numbering of the cyclopentanophenanthrene nucleus to the convention used by the IUPAC or Chemical Abstracts Service. The term "steroid" as used herein is intended to mean compounds having the aforementioned cyclopentanophenanthrene nucleus.



In these structures, the use of bold and dashed lines to denote particular conformation of groups again follows the IUPAC steroid-naming convention. The symbols "α" and "β" indicate the specific stereochemical configuration of a substituent at an asymmetric carbon atom in a chemical structure as drawn. Thus "α," denoted by a broken line, indicates that the group in question is below the general plane of the molecule as drawn, and "β," denoted by a bold line, indicates

that the group at the position in question is above the general plane of the molecule as drawn.

In addition, the five- and six-membered rings of the steroid molecule are often designated A, B, C and D as shown.

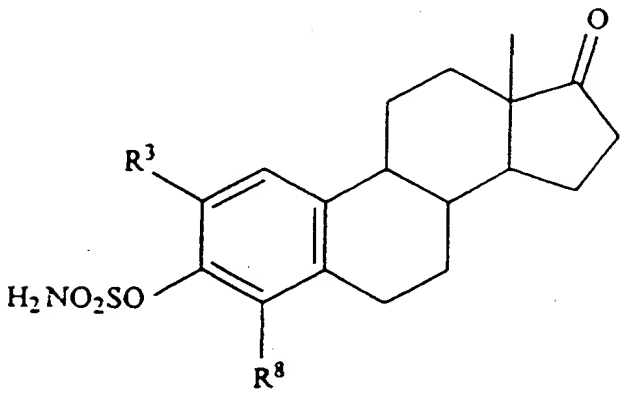
The Novel Compounds:

The novel compounds provided herein are those defined by structural formula (I), wherein R^1, R^2, R^3, R^8 through R^{12} and r1 are as defined above.

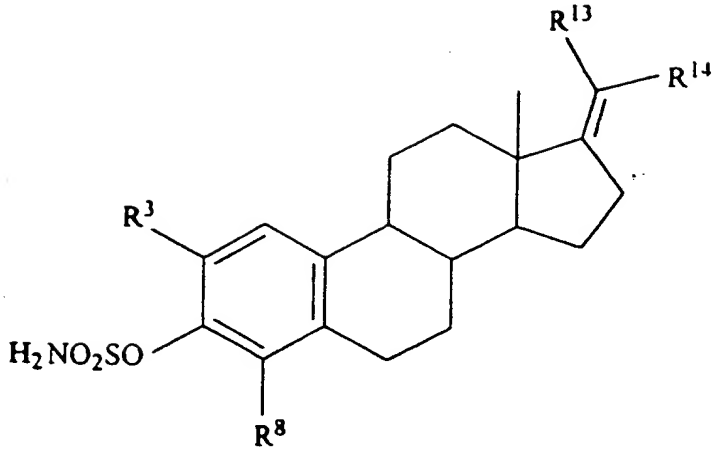
Preferred compounds are wherein R^1, R^2, R^9 and R^{10} are hydrogen, and the optional double bond r1 is not present.

Within this preferred group, particularly preferred compounds are:

(III)

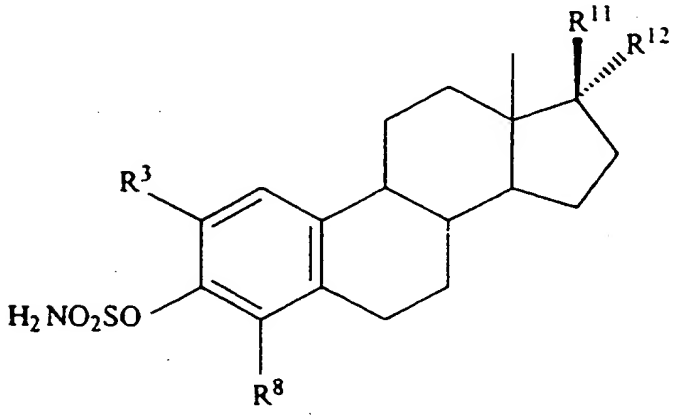


(IV)



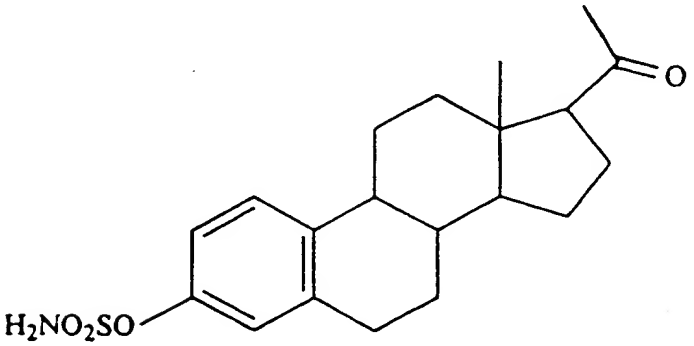
wherein R^3 and R^8 are as defined previously, and one of R^{13} and R^{14} is hydrogen, and the other is as defined previously, or wherein R^{13} and R^{14} are both -CN; and

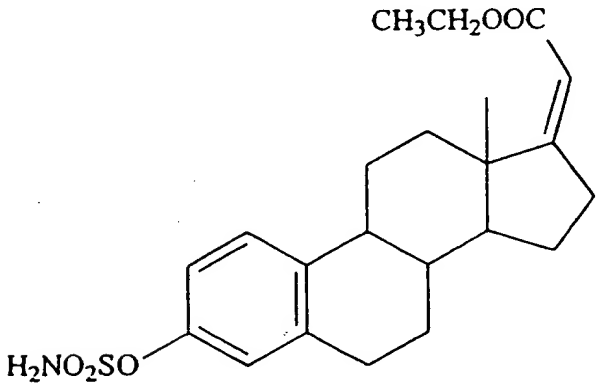
(V)

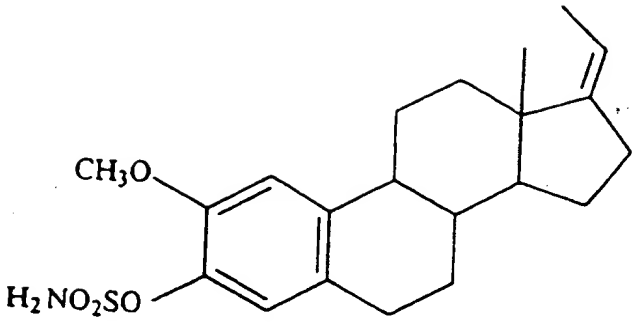


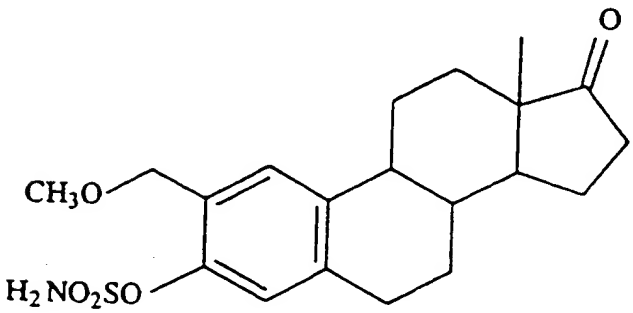
wherein one of R^{11} and R^{12} is hydrogen and the other is $-(CH_2)_m-O(CH_2)_q-N(CH_3)_2$, and wherein m is preferably 0 or 1 and q is preferably 2, 3 or 4.

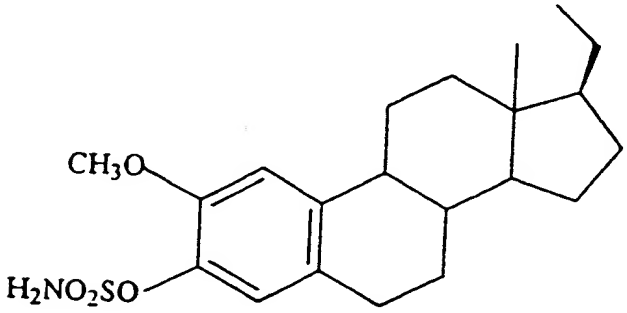
Examples of specific compounds of formula (I) include, but are not limited to, the following:

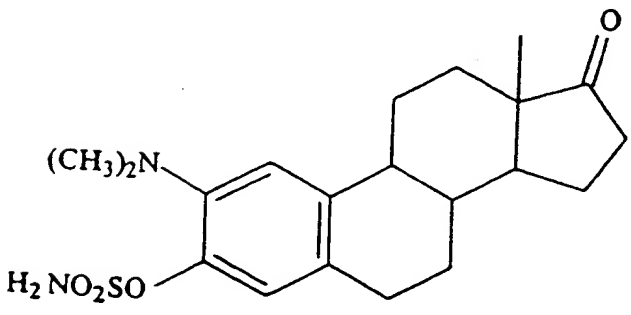


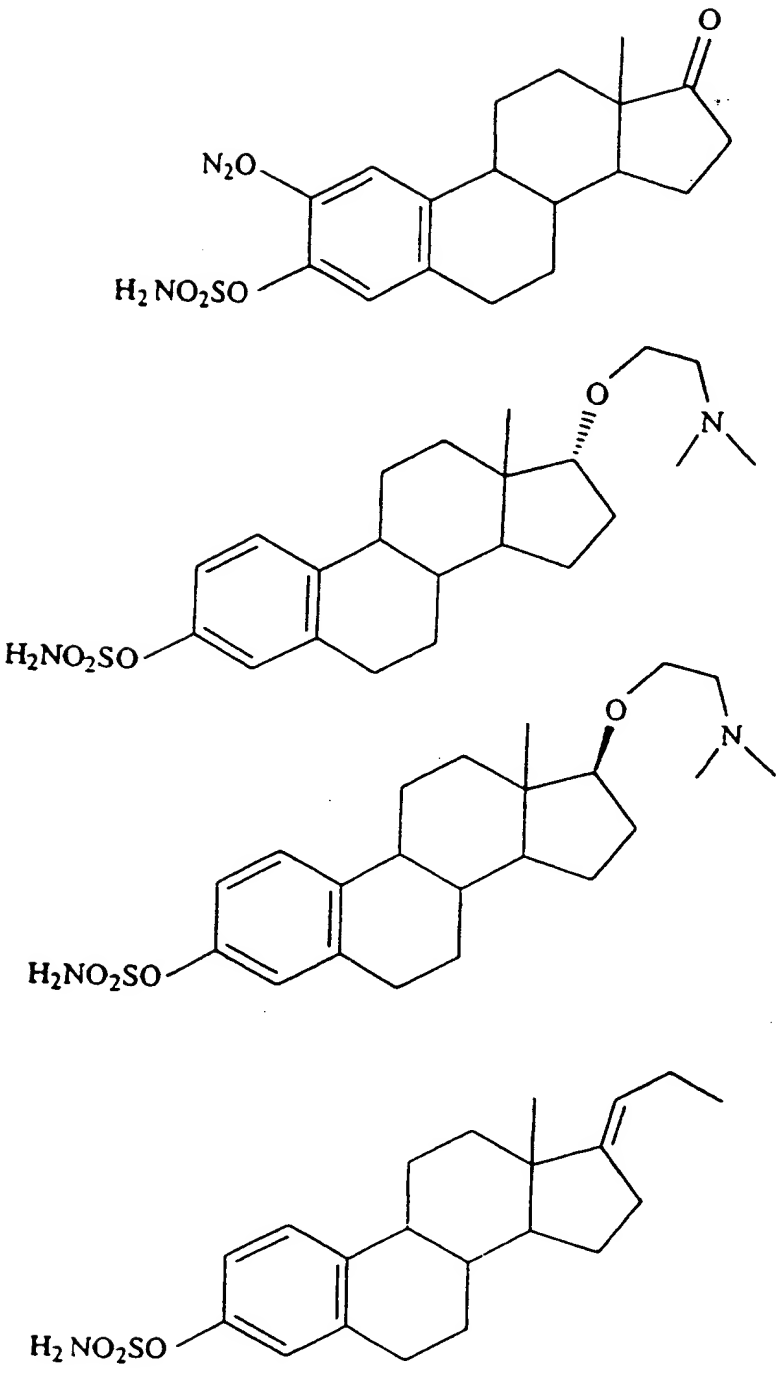


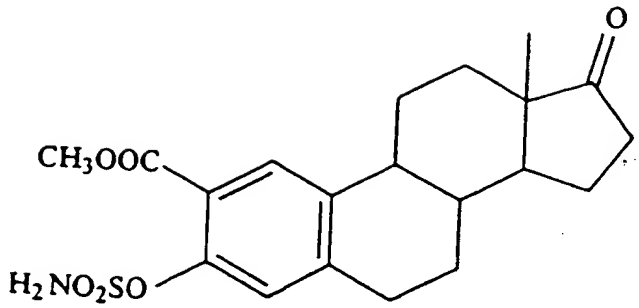

CH3O
H2NO2SO

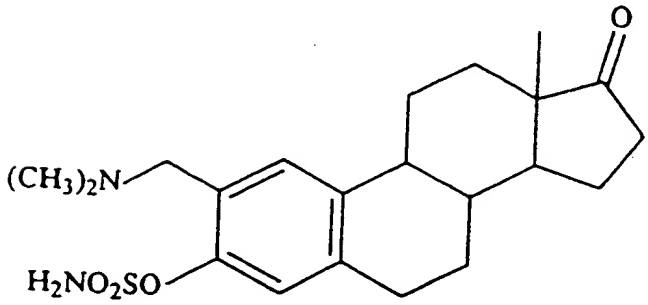

O
CH3O
H2NO2SO

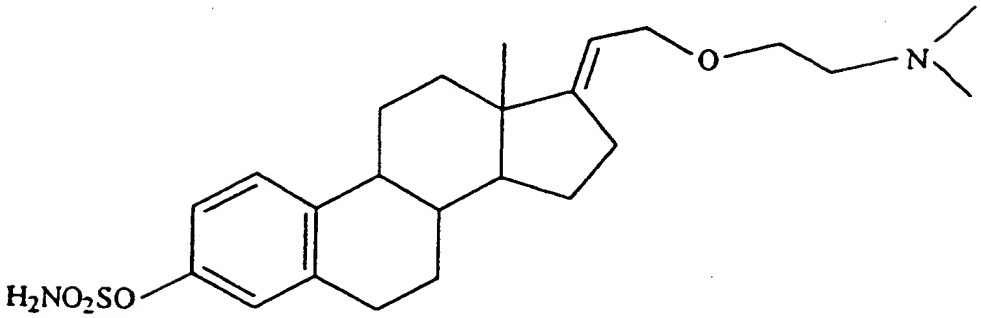

CH3O
H2NO2SO

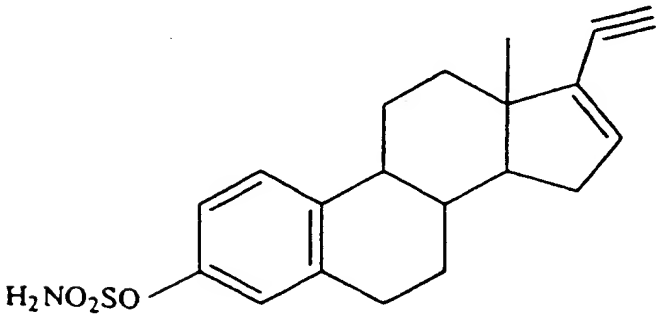

O
(CH3)2N
H2NO2SO

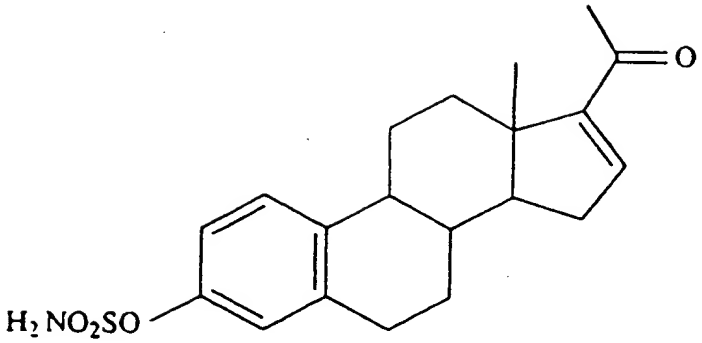

O
N2O
H2NO2SO
O
N
H2NO2SO
O
N
H2NO2SO
H2NO2SO

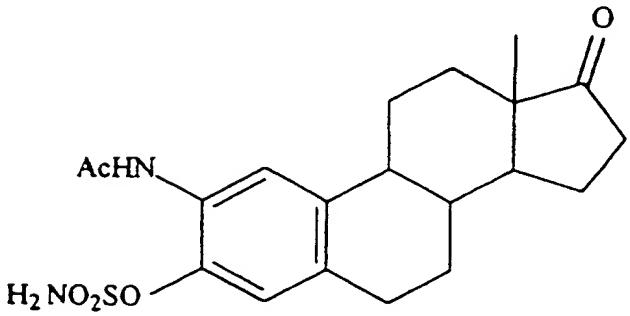

O
CH3OOC
H2NO2SO

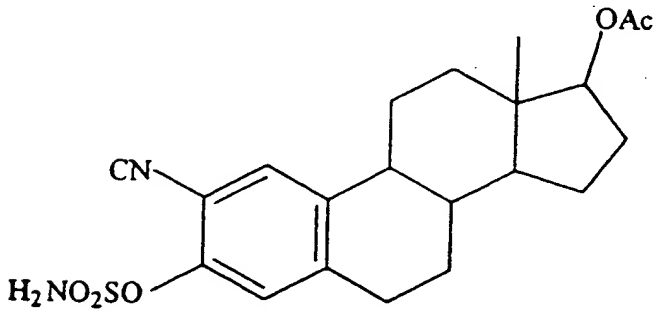

O
(CH3)2N
H2NO2SO

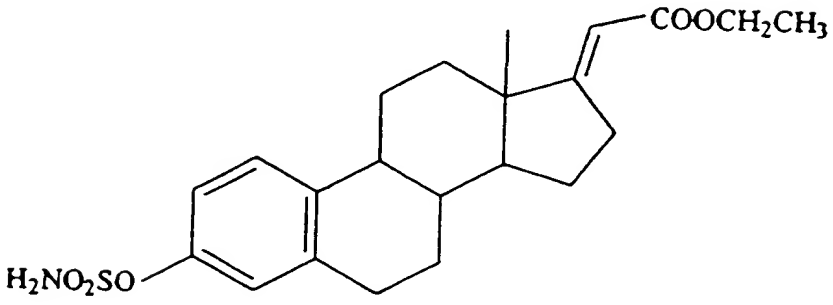

O
N
H2NO2SO

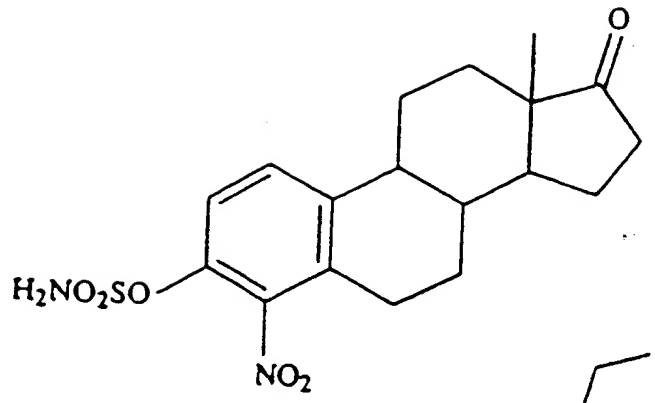

H2NO2SO

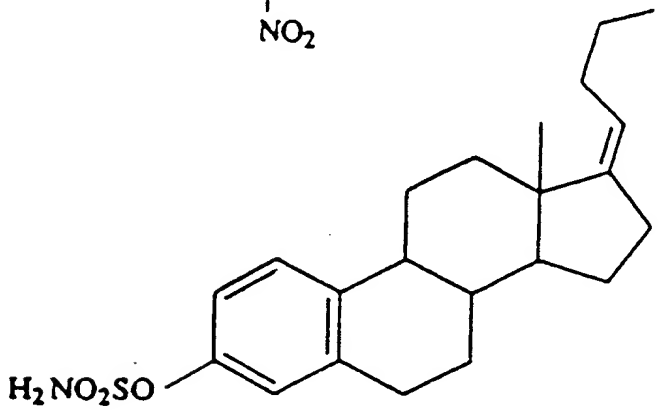

O
H_2NO_2SO

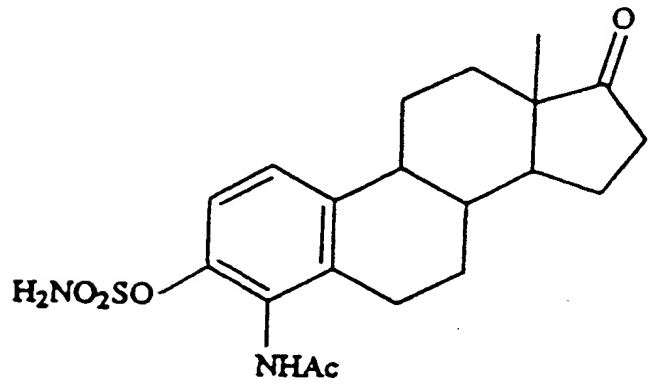

O
AcHN
H_2NO_2SO

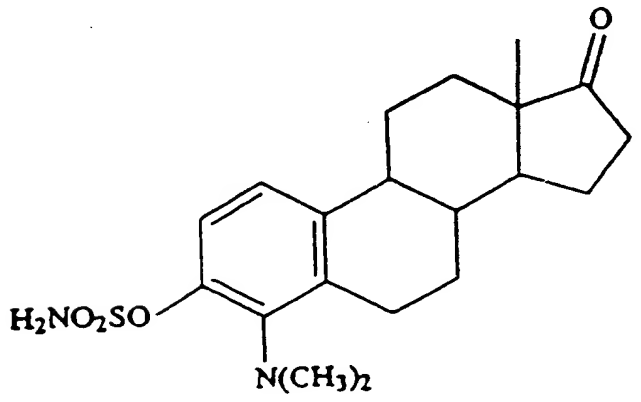

OAc
CN
H_2NO_2SO

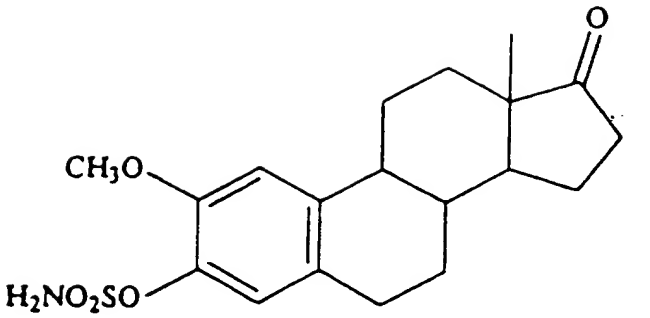

$COOCH_2CH_3$
H_2NO_2SO

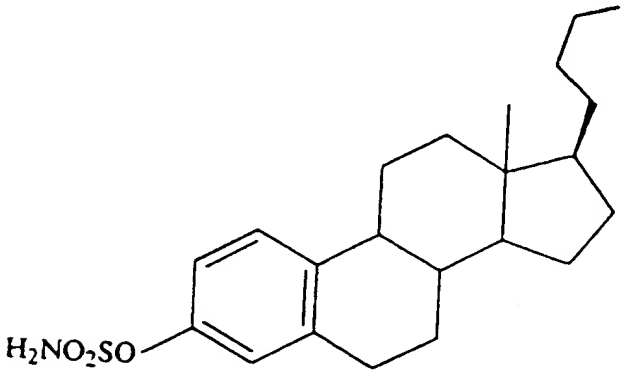

O
H_2NO_2SO
NO_2

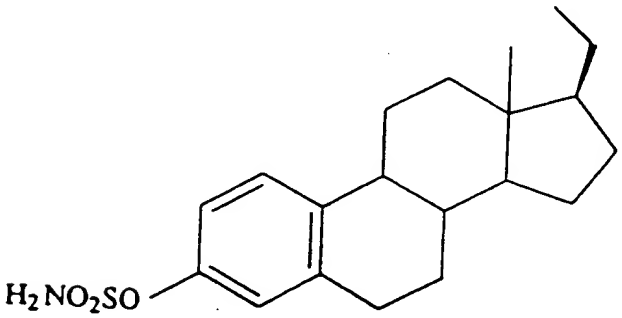

H_2NO_2SO

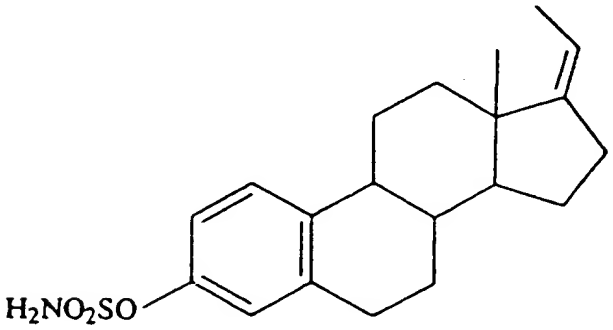

O
H_2NO_2SO
NHAc

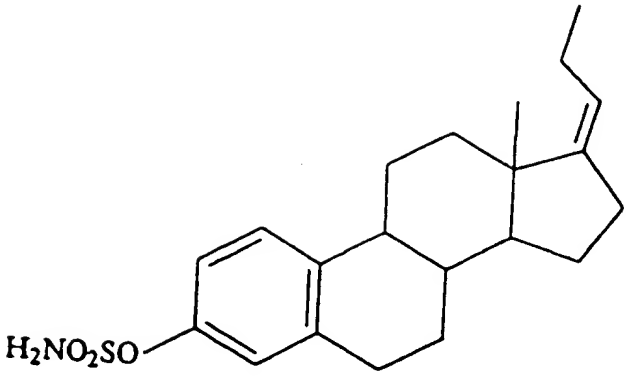

O
H_2NO_2SO
$N(CH_3)_2$

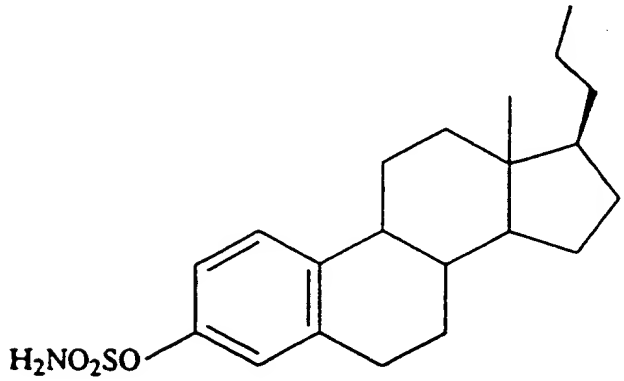

O
CH3O
H2NO2SO

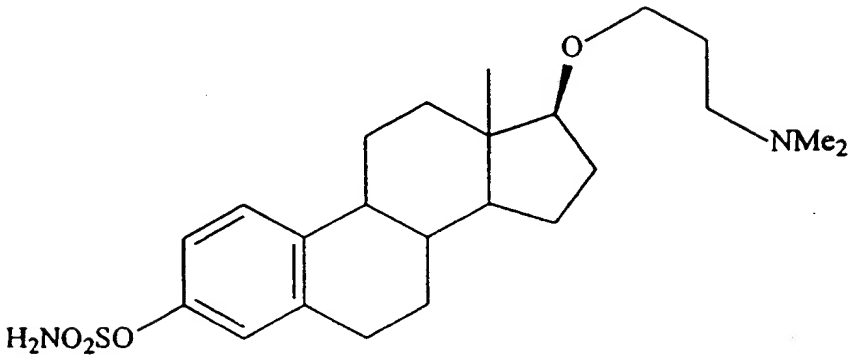

H2NO2SO

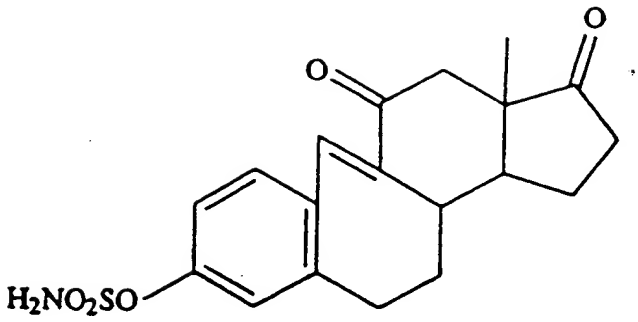

H2NO2SO

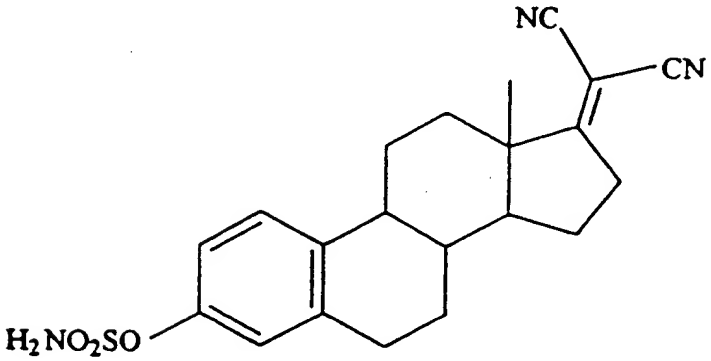

H2NO2SO


H_2NO_2SO


H_2NO_2SO


O
NMe_2
H_2NO_2SO





The compounds may be in the form of pharmaceutically acceptable salts or esters, or may be modified by appending one or more appropriate functionalities to enhance selected biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system,

increase oral bioavailability, increase solubility to allow administration by injection, and the like.

Salts of the compounds can be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, for example, by J. March, Advanced Organic Chemistry: Reactions, Mechanisms and Structure, 4th Ed. (New York: Wiley-Interscience, 1992). Acid addition salts are prepared from the free base (e.g., compounds having a neutral $-NH_2$ or cyclic amine group) using conventional means, involving reaction with a suitable acid. Typically, the base form of the compound is dissolved in a polar organic solvent such as methanol or ethanol and the acid is added at a temperature of about 0°C to about 100°C, preferably at ambient temperature. The resulting salt either precipitates or may be brought out of solution by addition of a less polar solvent. Suitable acids for preparing acid addition salts include both organic acids, e.g., acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. An acid addition salt may be reconverted to the free base by treatment with a suitable base.

Preparation of basic salts of acid moieties which may be present (e.g., carboxylic acid groups) are prepared in a similar manner using a pharmaceutically acceptable base such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, magnesium hydroxide, trimethylamine, or the like.

Preparation of esters involves functionalization of hydroxyl and/or carboxyl groups which may be present. These esters are typically acyl-substituted derivatives of free alcohol groups, i.e., moieties which are derived from carboxylic acids of the formula RCOOH where R is alkyl, and preferably is lower alkyl. Pharmaceutically acceptable esters may be prepared using methods known to those skilled in the art and/or described in the pertinent literature. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures.

Certain of the novel compounds are chiral in nature and can thus be in enantiomerically pure form or in a racemic mixture. The invention encompasses both the enantiomerically pure form of such compounds as well as diastereomeric and racemic mixtures thereof. Furthermore, certain compounds are stereoisomers which are asymmetric with respect to a C=C bond. In such a case, the invention encompasses both such structures, i.e., both the "E" and "Z" isomers, as well as mixtures thereof.

Utility and Administration:

The compounds defined by structural formula (I) are useful as estrone sulfatase inhibitors and are therefore useful for the treatment of estrogen-dependent disorders, i.e., conditions or diseases that are estrogen-induced or estrogen stimulated. Since the present compounds can lower circulating estrogen levels, they can effectively prevent the biologically active estrogens from reaching endocrine tumors. In addition, since the present compounds can reduce estrogen biosynthesis in tumors capable of endogenous estrogen synthesis, the present compounds are capable of inducing remissions in breast cancer, including metastatic tumors. Furthermore, the present compounds have utility in the treatment of ovarian, uterine and pancreatic tumors as well as disease conditions such as galactorrhea, McCurne-Albright syndrome, benign breast disease, and endometriosis.

An important feature of the preferred novel compounds described herein is that neither they nor their hydrolysis products are estrogenic. They are therefore especially advantageous for the applications described above because their administration will not exacerbate the conditions which they are used to treat.

In further preferred embodiments, the present compounds and their hydrolysis products are anti-estrogenic. Thus the compounds may be employed as anti-estrogenic agents, and are therefore useful for treating a variety of estrogen-dependent disorders, i.e., those conditions or diseases that are either induced or stimulated by estrogen. Such conditions include, but are not limited to: breast cancer, including metastatic tumors; ovarian, uterine and pancreatic tumors; and

disease conditions such as galactorrhea, McCurne-Albright syndrome, benign breast disease, and endometriosis.

The compounds may be conveniently formulated into pharmaceutical compositions composed of one or more of the compounds in association with a pharmaceutically acceptable carrier. See Remington: The Science and Practice of Pharmacy, 19th Ed. (Easton, PA: Mack Publishing Co., 1995), which discloses typical carriers and conventional methods of preparing pharmaceutical compositions which may be used to prepare formulations using the novel enzyme inhibitors of the invention. The compounds may also be administered in the form of pharmaceutically acceptable salts, or in the form of pharmaceutically acceptable esters, as explained in the preceding section.

The compounds may be administered orally, parenterally, transdermally, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term "parenteral" as used herein is intended to include subcutaneous, intravenous, and intramuscular injection. The amount of active compound administered will, of course, be dependent on the subject being treated, the subject's weight, the manner of administration and the judgment of the prescribing physician. Generally, however, dosage will be in the range of approximately 0.01 mg/kg/day to 10.0 mg/kg/day, more preferably in the range of about 1.0 mg/kg/day to 5.0 mg/kg/day.

Depending on the intended mode of administration, the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include, as noted above, an effective amount of the selected inhibitor in combination with a pharmaceutically acceptable carrier and, in addition, may include other pharmaceutical agents, adjuvants, diluents, buffers, etc.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate,

sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc., an estrone sulfatase inhibitor as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington: The Science and Practice of Pharmacy, referenced above.

For oral administration, the composition will generally take the form of a tablet or capsule, or may be an aqueous or nonaqueous solution, suspension or syrup. Tablets and capsules are preferred oral administration forms. Tablets and capsules for oral use will generally include one or more commonly used carriers such as lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. When liquid suspensions are used, the active agent is combined with emulsifying and suspending agents. If desired, flavoring, coloring and/or sweetening agents may be added as well. Other optional components for incorporation into an oral formulation herein include, but are not limited to, preservatives, suspending agents, thickening agents, and the like.

Parenteral administration, if used, is generally characterized by injection. Injectable formulations can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Preferably, sterile injectable suspensions are formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable formulation may also be a sterile injectable solution or a suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In

addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795.

The compounds of the invention may also be administered through the skin or mucosal tissue using conventional transdermal drug delivery systems, wherein the agent is contained within a laminated structure that serves as a drug delivery device to be affixed to the skin. In such a structure, the drug composition is contained in a layer, or "reservoir," underlying an upper backing layer. The laminated structure may contain a single reservoir, or it may contain multiple reservoirs. In one embodiment, the reservoir comprises a polymeric matrix of a pharmaceutically acceptable contact adhesive material that serves to affix the system to the skin during drug delivery. Examples of suitable skin contact adhesive materials include, but are not limited to, polyethylenes, polysiloxanes, polyisobutylenes, polyacrylates, polyurethanes, and the like. Alternatively, the drug-containing reservoir and skin contact adhesive are present as separate and distinct layers, with the adhesive underlying the reservoir which, in this case, may be either a polymeric matrix as described above, or it may be a liquid or hydrogel reservoir, or may take some other form.

The backing layer in these laminates, which serves as the upper surface of the device, functions as the primary structural element of the laminated structure and provides the device with much of its flexibility. The material selected for the backing material should be selected so that it is substantially impermeable to the active agent and any other materials that are present. The backing layer may be either occlusive or nonocclusive, depending on whether it is desired that the skin become hydrated during drug delivery. The backing is preferably made of a sheet or film of a preferably flexible elastomeric material. Examples of polymers that are suitable for the backing layer include polyethylene, polypropylene, polyesters, and the like.

During storage and prior to use, the laminated structure includes a release liner. Immediately prior to use, this layer is removed from the device to expose the

basal surface thereof, either the drug reservoir or a separate contact adhesive layer, so that the system may be affixed to the skin. The release liner should be made from a drug/vehicle impermeable material.

Transdermal drug delivery devices may be fabricated using conventional techniques, known in the art, for example by casting a fluid admixture of adhesive, drug and vehicle onto the backing layer, followed by lamination of the release liner. Similarly, the adhesive mixture may be cast onto the release liner, followed by lamination of the backing layer. Alternatively, the drug reservoir may be prepared in the absence of drug or excipient, and then loaded by soaking in a drug/vehicle mixture.

The laminated transdermal drug delivery systems may in addition contain a skin permeation enhancer. That is, because the inherent permeability of the skin to some drugs may be too low to allow therapeutic levels of the drug to pass through a reasonably sized area of unbroken skin, it is necessary to coadminister a skin permeation enhancer with such drugs. Suitable enhancers are well know in the art and include, for example, dimethylsulfoxide (DMSO), dimethyl formamide (DMF), N,N-dimethylacetamide (DMA), decylmethylsulfoxide (C_{10}MSO), C_2-C_6 alkanediols, and the 1-substituted azacycloheptan-2-ones, particularly 1-n-dodecylcyclaza-cycloheptan-2-one (available under the trademark Azone® from Whitby Research Incorporated, Richmond, VA), alcohols, and the like.

Alternatively, the pharmaceutical compositions of the invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of the invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable

preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

Preferred formulations for vaginal drug delivery are ointments and creams. Ointments are semisolid preparations which are typically based on petrolatum or other petroleum derivatives. Creams containing the selected active agent, are, as known in the art, viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase, also sometimes called the "internal" phase, is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation is generally a nonionic, anionic, cationic or amphoteric surfactant. The specific ointment or cream base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing. Also preferred are vaginal suppositories. Suppositories may be formulated using conventional means, e.g., compaction, compression-molding or the like, and will contain carriers suited to vaginal drug delivery, typically a bioerodible material which provides for the desired drug release profile.

Formulations for buccal administration include tablets, lozenges, gels and the like. Alternatively, buccal administration can be effected using a transmucosal delivery system.

Process for Preparation:

The compounds of the invention may be prepared in high yield using relatively simple, straightforward methods as exemplified in the experimental section herein. Syntheses of representative compounds are detailed in Examples 1 through 32. Reference may also be had to co-pending, commonly assigned U.S. Patent Application Serial No. 08/998,877, entitled "Novel Anti-Estrogenic Steroids,

and Associated Pharmaceutical Compositions and Methods of Use," inventors Tanabe et al.(filed on even date herewith.)

Experimental

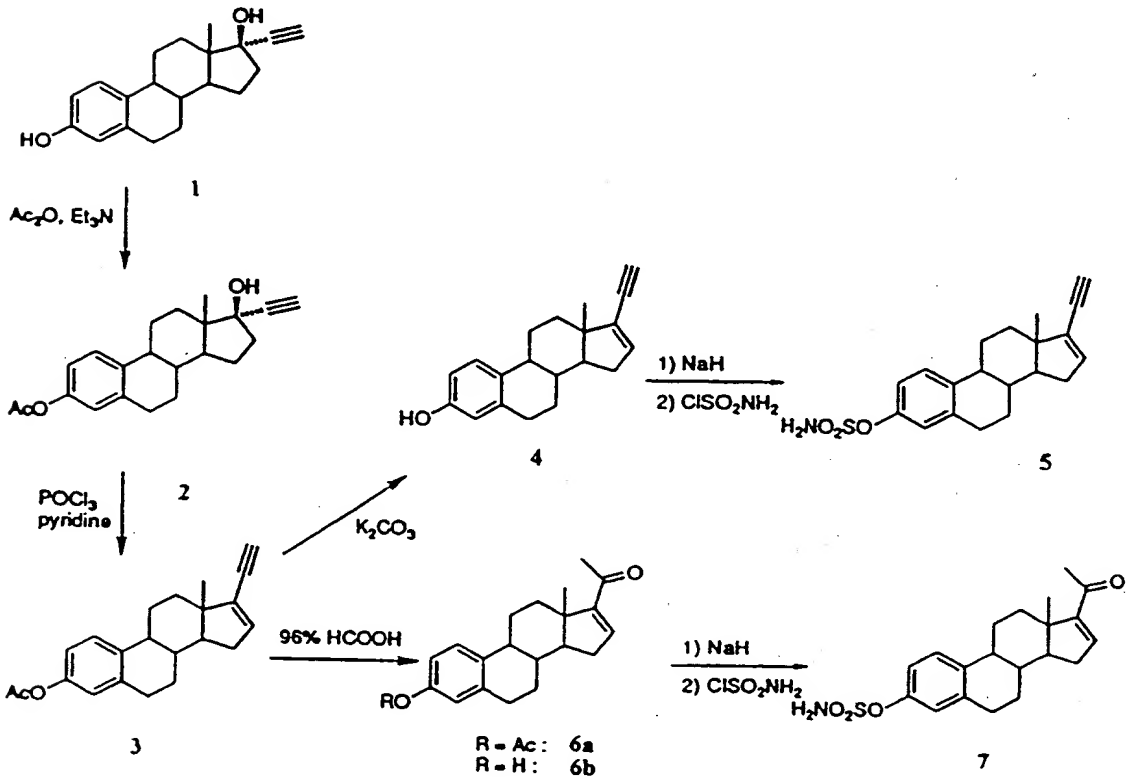
The practice of the present invention will employ, unless otherwise indicated, conventional techniques of synthetic organic chemistry, biological testing, and the like, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Fieser et al., Steroids (New York: Reinhold, 1959), Djerassi, Steroid Reactions: An Outline for Organic Chemists (San Francisco: Holden-Day, 1963), and Fried et al., Organic Reactions in Steroid Chemistry, vols. 1 and 2 (New York: Reinhold, 1972), for detailed information concerning steroid-related synthetic procedures. Reference may be had to MacIndoe et al., *Endocrinology* 123(3):1281-1287 (1988), Duncan et al., *Cancer Res.* 53:298-303 (1993), and Yue et al., *J. Steroid Biochem.* 44:671-673 (1993), for a description of the biological testing procedures useful to evaluate compounds such as those described and claimed herein.

It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that the description above as well as the examples which follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

In the following examples, efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental error and deviation should be accounted for. Unless indicated otherwise, temperature is in degrees C and pressure is at or near atmospheric. All solvents were purchased as HPLC grade, and all reactions were routinely conducted under an inert atmosphere of argon unless otherwise indicated. All reagents were obtained commercially unless otherwise indicated. Estrone and estradiol were purchased from Berlichem U.S.; ethynyl estradiol was purchased from Akzo Nobel. NMR

analyses were conducted on either a Varian Gemini 300 and were referenced to chloroform at δ 7.27. FTIR spectra were recorded on a Perkin-Elmer 1610.

The following scheme illustrates the synthetic steps carried out in Examples 1 and 2 to make the estrone sulfatase inhibitors (5) and (7):



Scheme 1

Example 1

Preparation of 19-Norpregna-1,3,5(10),16-tetraen-20-yne-3-*O*-sulfamate (5)

(a) Synthesis of Ethynylestradiol 3-*O*-acetate (2):

To a solution of ethynylestradiol (1, 5.92 g, 20 mmol) in tetrahydrofuran (THF) (30 mL) and CH_2Cl_2 (70 mL) were added triethylamine (6.8 ml, 50 mmol) and acetic anhydride (2.8 ml, 30 mmol); the mixture was then stirred for 17 h at room temperature. Saturated aqueous NH_4Cl was added to the reaction mixture, which was then extracted with ethyl acetate (EtOAc). The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 6.08 g of 2 (90% yield) mp: 147-148 °C.

^{1}H NMR: δ 7.29 (d, 1H, aromatic), 6.90-6.75 (m, 2H, aromatic), 2.60 (s, 1H, -C≡CH), 2.28 (s, 3H, -OCOCH$_3$), 0.88 (s, 3H, 18-CH$_3$).

(b) Synthesis of 19-Norpregna-1,3,5(10),16-tetraen-20-yn-3-ol 3-*O*-acetate (3):

To a solution of ethynylestradiol 3-*O*-acetate (2, 3.05 g, 9.0 mmol) in pyridine (25 mL) was added phosphorousoxychloride (1.7 ml, 18 mmol) and stirred for 2 h at 110°C. Afterwards, the reaction mixture was cooled to room temperature, poured into ice (100g) and acidified with 5 N HCl, and then extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (15:1~10:1, v/v) to afford 1.83 g of 3 (64% yield) mp: 104-106°C.

^{1}H NMR: δ 7.28 (d, 1H, aromatic), 6.95-6.75 (m, 2H aromatic), 6.22-6.10 (m, 1H, 16-H), 3.09 (s, 1H, -C≡CH), 2.28 (s, 3H, -OCOCH$_3$), 0.88 (s, 3H, 18-CH$_3$).

(c) Synthesis of 19-Norpregna-1,3,5(10),16-tetraen-20-yn-3-ol (4):

To a solution of 19-norpregna-1,3,5(10),16-tetraen-20-yn-3-ol 3-*O*-acetate (3, 0.670 g, 2.1 mmol) in THF (2.0 mL) and methanol (MeOH) (5.0 mL) was added potassium carbonate (0.290 g, 2.1 mmol) and stirred for 1 h at room temperature. The reaction mixture was acidified with 1 N HCl, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1–2:1, v/v) to afford 0.573 g of (4) (99% yield) mp: 158-159°C.

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.68-6.52 (m, 2H, aromatic), 6.20-6.10 (m, 1H, 16-H), 4.55 (s, 1H, -OH), 3.09 (s, 1H, -C≡CH), 0.88 (s, 3H, 18-CH_3); MS (EI): *m/z* 278 (M^+).

(d) Synthesis of 19-Norpregna-1,3,5(10),16-tetraen-20-yne-3-*O*-sulfamate (5):

To a solution of chlorosulfonyl isocyanate (0.22 ml, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 ml of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 19-norpregna-1,3,5(10),16-tetraen-20-yn-3-ol (4, 0.139 g, 0.5 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was then added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica el) using n-hexane:EtOAc (3:1–2:1, v/v) to afford 0.142 g of 5, (79% yield) mp: 210°C (decomposed).

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.15-7.00 (m, 2H, aromatic), 6.18-6.12 (m, 1H, 16-H), 4.91 (s, 2H, -NH_2), 3.09 (s, 1H, -C≡CH), 0.88 (s, 3H, 18-CH_3); MS (EI): *m/z* 357 (M^+).

Example 2

Preparation of 19-Norpregna-1,3,5(1),16-tetraen-20-one-3-*O*-sulfamate (7)

(a) Synthesis of 19-Norpregna-1,3,5(10),16-tetraen-20-yn-3-ol 3-*O*-acetate (**3**):

The procedure described in steps (a) and (b) of Example 1 above was used to obtain **3**.

(b) Synthesis of 3-Hydroxy-19-norpregna-1,3,5(10,16-tetraen-20-one 3-*O*-acetate (**6a**) and 3-Hydroxy-19-norpregna-1,3,5(10),16-tetraen-20-one (**6b**):

A solution of 19-norpregna-1,3,5(10),16- tetraen-20-yn-3-ol 3-*O*-acetate (**3**, 1.05 g, 3.3 mmol) in 96% formic acid (30 mL) was stirred for 30 min at 100°C. Afterwards, the reaction mixture was cooled to room temperature, poured into ice (100 g) and stood for 18 h at 0°C. The precipitate was collected by filtration and washed with H_2O, and purified by column chromatography (silica gel) using n-hexane:EtOAc (5:16–2:1, v/v) to afford 0.418 g of **6a** (37% yield) mp: 154-155°C, and 0.267 g of **6b** (27% yield) mp: 243-244°C.

6a: ^{1}H NMR: δ 7.28 (d, 1H, aromatic), 6.95-6.68 (m, 3H, aromatic, 16-H), 2.28 (s, 3H, 21-CH_3), 2.28 (s, 3H, -OCOCH_3), 0.92 (s, 3H, 18-CH_3).

6b: ^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.80-6.72 (m, 1H, 16-H), 6.68-6.53 (m, 2H, aromatic), 2.29 (s, 3H, 21-CH_3), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 296 (M^+).

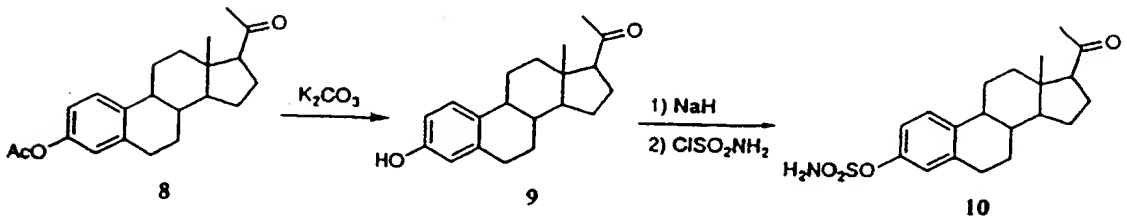
(c) Synthesis of 19-Norpregna-1,3,5(10),16-tetraen-20-one-3-*O*-sulfamate (**7**):

Beginning with 3-hydroxy-19-norpregna- 1,3,5(10),16-tetraen-20-one (**6b**, 0.148 g, 0.50 mmol), by using the procedure described in step (d) of Example 1 above, 0.139 g of (**7**) (74% yield) mp: 189-190°C was obtained after chromatography (n-hexane:EtOAc 2:1–3:2, v/v).

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.13-7.00 (m, 2H, aromatic), 6.78-6.70 (m, 1H, 16-H), 4.97 (s, 2H, -NH_2), 2.29 (s, 3H, 21-CH_3), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 375 (M^+).

Example 3

Preparation of 19-Norpregna-1,3,5(10)trien-20-one-3-*O*-sulfamate (10)



Scheme 2

(a) Synthesis of 3-Hydroxy-19-norpregna-1,3,5(10)trien-20-one (9):

To a solution of 3-hydroxy-19-norpregna- 1,3,5(10)trien-20-one 3-*O*-acetate (8, 0.340 g, 1.0 mmol) in THF (5.0 mL) and MeOH (5.0 mL) was added potassium carbonate (0.138 g, 1.0 mmol) at 0°C. The reaction mixture was stirred for 2 h, and quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 0.267 g of 9 (90% yield) mp: 237-238°C.

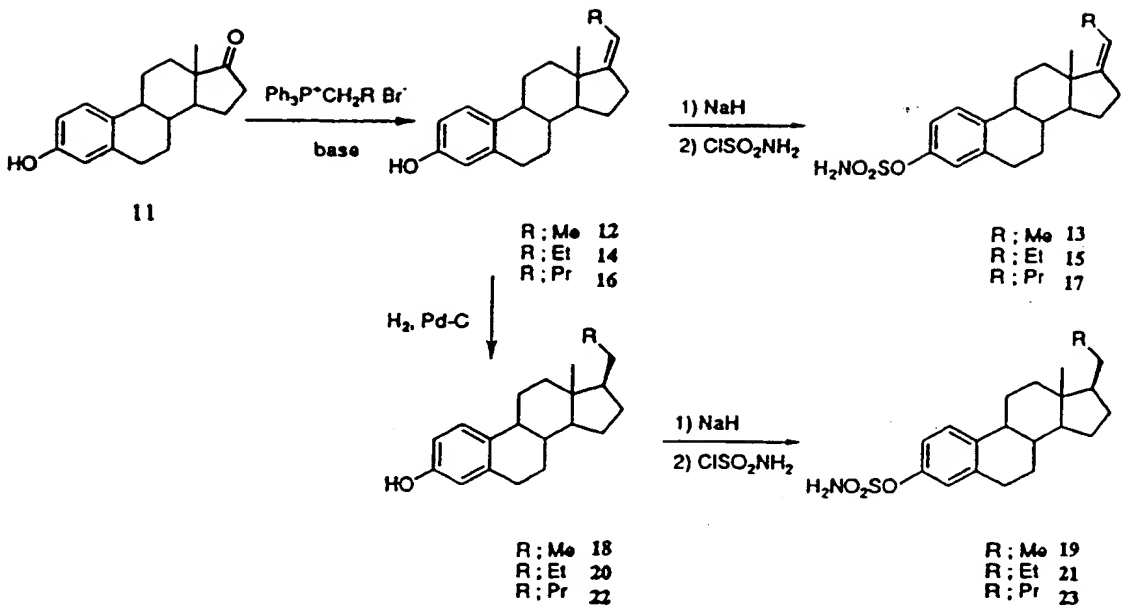
^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.67-6.52 (m, 2H, aromatic), 4.76 (s, 1H, -OH), 2.62 (t, 1H,17α-H), 2.16 (s, 3H, 21-CH_3), 0.66 (s, 3H, 18-CH_3); MS (EI): *m/z* 298 (M^+).

(b) Synthesis of 19-Norpregna-1,3,5(10)trien-20- one-3-*O*-sulfamate (**10**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-hydroxy-19-norpregna-1,3,5(10)trien-20-one (**9**, 0.149 g, 0.5 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 0.151 g of **10** (80% yield) mp: 189-190°C.

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.13-7.00 (m, 2H, aromatic), 4.89 (s, 2H, $-NH_2$), 2.61 (t, 1H,17α-H), 2.16 (s, 3H, 21-CH_3), 0.66 (s, 3H, 18-CH_3); MS (EI): *m/z* 377 (M^+).

The following scheme illustrates the synthetic steps carried out in Examples 4 through 9 to make the anti-estrogenic compounds (**13**), (**15**), (**17**), (**19**), (**21**) and (**23**):



11

Scheme 3

Example 4

Synthesis of [17(20)Z]-19-Norpregna-1,3,5(10),17(20)-tetraene-3-*O*-sulfamate (13)

(a) Synthesis of [17(20)Z]-19-Norpregna-1,3,5(10),17,(20)-tetraen-3-ol (**12**):

To a suspension of ethyltriphenylphosphonium bromide (4.64 g, 12.5 mmol) in THF (40 mL) was added potassium *tert*-butoxide (1.35 g, 12 mmol) and stirred for 30 min at room temperature. Estrone (**11**, 1.35 g, 5.0 mmol) was then added, and the mixture was then stirred for 24 h at room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column

chromatography (silica gel) using n-hexane:EtOAc (5:1-2:1, v/v) to afford 1.03 g of **12** (72% yield) mp: 138-139°C.

^{1}H NMR: δ 7.16 (d, 1H, aromatic), 6.68-6.50 (m, 2H, aromatic), 5.22-5.08 (m, 1H, =C<u>H</u>-CH$_3$), 4.48 (s, 1H, -OH), 1.72-1.65 (m, 3H, =CH-C<u>H</u>$_3$), 0.91 (s, 3H, 18-CH$_3$); MS (EI): *m/z* 282 (M^+).

(b) Synthesis of [17(20)Z]-19-Norpregna-1,3,5(10),17(20)-tetraene-3-*O*-sulfamate (**13**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of [17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraene-3-ol (**12**, 0.141 g, 0.50 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:$CHCl_3$:EtOAc (5:5:1-3:3:1, v/v/v) to afford 0.181 g of **13** (100% yield) mp: 131-132°C.

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.18-6.95 (m, 2H, aromatic), 5.25-5.10 (m, 1H, =CH-CH$_3$), 4.96 (s, 2H, -NH_2), 1.69 (d, 3H, =CH-C<u>H</u>$_3$), 0.91 (s, 3H, 18-CH$_3$); MS (DCI): *m/z* 379 ($M^+ + NH_4^+$), 362 ($M^+ + H$).

<u>Example 5</u>

<u>Synthesis of [17(2))Z]-Propylideneestra-1,3,5(10)-triene-3-*O*-sulfamate (15)</u>

(a) Synthesis of [17(20)Z]-Propylideneestra-1,3,5(1)-trien-3-ol (**14**):

To sodium hydride (1.20 g of a mineral oil dispersion, 60%, 30 mmol) was added DMSO (100 mL) and the mixture stirred for 1 h at 75°C. Propyltriphenyl-phosphonium bromide (12.3 g, 32.0 mmol) was then added, and stirring continued

for 30 min at room temperature. Estrone (**11**, 2.70 g, 10 mmol) was added to the reaction mixture, and it was then stirred for 4 days at 80°C. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C and extracted with Et_2O. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (10:1→1:1, v/v) to afford 1.30 g of **14** (44% yield) and 1.05 g of the starting material **11** (39% yield) mp: 149-151°C.

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.68-6.50 (m, 2H, aromatic), 5.12-5.00 (m, 1H, =C<u>H</u>-CH_2-), 4.57 (s, 1H, -OH), 0.96 (t, 3H, 23-CH_3), 0.90 (s, 3H, 18-CH_3); MS (EI): *m/z* 296 (M^+).

(b) Synthesis of [17(20)Z]-Propylideneestra-1,3,5(10)-triene-3-*O*-sulfamate (**15**):

Beginning with [17(20)Z]-propylideneestra-1,3,5(10)-trien-3-ol (**14**, 0.148 g, 0.50 mmol), by using the procedure described in step (b) of Example 4 above, 0.145 g of **15** (77% yield; mp: 120-121°C) was obtained after chromatography (n-hexane:EtOAc 5:1→2:1, v/v).

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.13-6.98 (m, 2H, aromatic), 5.12-5.00 (m, 1H, =C<u>H</u>-CH_2-), 4.94 (s, 2H, -NH_2), 0.96 (t, 3H, 23-CH_3), 0.90 (s, 3H, 18-CH_3); MS (EI): *m/z* 375 (M^+).

Example 6

Preparation of [17(20)]-19,21-Dinorchola-1,3,5(10)17(20)-tetraene-3-*O*-sulfamate (17)

(a) Synthesis of [17(20)Z]-19,21-Dinorchola-1,3,5(10)17(20)-tetraen-3-ol (**16**):

To a suspension of butyltriphenylphosphonium bromide (12.8 g, 32.0 mmol) in THF (100 mL) was added potassium *tert*-butoxide (3.37 g, 30 mmol) and stirred for 30 min at room temperature. Estrone (**11**, 2.70 g, 10 mmol) was added to the reaction mixture, and stirring continued for 5 days at 80°C. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C and extracted with EtOAc.

The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (10:1~2:1, v/v) to afford 2.45 g of **16** (79% yield) mp: 85-86°C.

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.70-6.52 (m, 2H, aromatic), 5.13-5.00 (m, 1H, =C<u>H</u>-CH_2-), 4.49 (s, 1H, -OH), 0.91 (t, 3H, 24-CH_3), 0.90 (s, 3H, 18-CH_3); MS (EI): *m/z* 310 (M^+).

(b) Synthesis of [17(20)Z]-19,21-Dinorchola-1,3,5(10)17(20)-tetraene-3-*O*-sulfamate (**17**):

Beginning with [17(20)Z]-19,21- dinorchola-1,3,5(10)17(20)-tetraen-3-ol (**16**, 0.176 g, 0.57 mmol), by using the procedure described in step (b) of Example 4 above, 0.173 g of **17** (78% yield; mp: 117-118°C) was obtained after chromatography (n-hexane:EtOAc 5:1~3:1, v/v).

^{1}H NMR: δ 7.30 (d, 1H, aromatic), 7.15-6.97 (m, 2H, aromatic), 5.20-4.85 (m, 3H, =C<u>H</u>-CH_2-, and -NH_2), 0.91 (t, 3H, 24-CH_3), 0.90 (s, 3H, 18-CH_3); MS (EI): *m/z* 389 (M^+).

Example 7

Preparation of 19-Norpregna-1,3,5(10)-triene-3-*O*-sulfamate (19)

(a) Synthesis of [17(20)Z]-19-Norpregna-1,3,5(10),17,(20)-tetraen-3-ol (**12**):

By using the procedure described in step (a) of Example 4 above, **12** was obtained from **11**.

(b) Synthesis of 19-Norpregna-1,3,5(10)-trien-3-*O*-ol (**18**):

To a solution of [17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**12**, 0.565 g, 2.00 mmol) in EtOAc (10 mL) was added 10% palladium on carbon (0.100 g). The reaction mixture was stirred for 2 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel)

using n-hexane:EtOAc (5:1, v/v) to afford 0.483 g of **18** (85% yield) mp: 112-113°C.

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.67-6.50 (m, 2H, aromatic), 0.90 (t, 3H, 21-CH_3), 0.60 (s, 3H, 18-CH_3); MS (EI): *m/z* 284 (M^+).

(c) Synthesis of 19-Norpregna-1,3,5(10)-triene-3-*O*-sulfamate (**19**):

Beginning with 19-norpregna-1,3,5(10)- trien-3-ol (**18**, 0.284 g, 1.00 mmol), by using the procedure described in step (b) of Example 4 above, 0.279 g of **19** (77% yield; mp: 167-168°C) was obtained after chromatography (n-hexane:EtOAc 3:1→2:1, v/v).

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.12-6.98 (m, 2H, aromatic), 4.90 (s, 2H, -NH_2), 0.90 (t, 3H, 21-CH_3), 0.61 (s, 3H, 18-CH_3); MS (DCI): *m/z* 381 (M^++NH_4^+); HRMS calcd. for $C_{20}H_{28}N_1O_3S_1$, 362.1790; found, 362.1812.

Example 8

Preparation of 17β-Propylestra-1,3,5(10)-triene-3-*O*-sulfamate (21)

(a) Synthesis of [17(20)Z]-Propylideneestra-1,3,5(1)-trien-3-ol (**14**):

By using the procedure described in step (a) of Example 5 above, **14** was obtained from **11**.

(b) Synthesis of 17β-Propylestra-1,3,5(10)-trien- 3-ol (**20**):

Beginning with [17(20)Z]-propylideneestra-1,3,5(10)-trien-3-ol (**14**, 0.371 g, 1.25 mmol), by using the procedure described in step (b) of Example 7 above, 0.311 g of **20** (83% yield; mp: 130-131°C) was obtained after chromatography (n-hexane:EtOAc 10:1→5:1, v/v).

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.70-6.52 (m, 2H, aromatic), 4.49 (s, 1H, -OH), 0.90 (t, 3H, 23-CH_3), 0.60 (s, 3H, 18-CH_3); MS (EI): *m/z* 298 (M^+).

(c) Synthesis of 17β-Propylestra-1,3,5(10)-triene-3-*O*-sulfamate (**21**):

Beginning with 17β-propylestra-1,3,5(10)-trien-3-ol (**20**, 0.149 g, 0.50 mmol), by using the procedure described in step (b) of Example 4 above, 0.184 g of **21** (97% yield; mp: 170-171°C) was obtained after chromatography (n-hexane:EtOAc 5:1→2:1, v/v).

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.15-7.00 (m, 2H, aromatic), 4.89 (s, 2H, -NH_2), 0.91 (t, 3H, 23-CH_3), 0.61 (s, 3H, 18-CH_3); MS (EI): *m/z* 377 (M^+).

Example 9

Preparation of 19,21-Dinorchola-1,3,5(10)-triene-3-*O*-sulfamate (23)

(a) Synthesis of [17(20)Z]-19,21-Dinorchola- 1,3,5(10)17(20)-tetraen-3-ol (**16**):

By using the procedure described in step (a) of Example 6 above, **16** was obtained from **11**.

(b) Synthesis of 19,21-Dinorchola-1,3,5(10)-trien-3-ol (**22**):

Beginning with [17(20)Z]-19,21-dinorchola-1,3,5(10)17(20)-tetraen-3-ol (**16**, 0.473 g, 1.52 mmol), 0.367 g of **22** (77% yield; mp: 97-98°C) was obtained after chromatography (n-hexane:EtOAc 10:1, v/v).

^{1}H NMR: δ 7.16 (d, 1H, aromatic), 6.70-6.50 (m, 2H, aromatic), 4.56 (s, 1H, -OH), 0.90 (t, 3H, 24-CH_3), 0.61 (s, 3H, 18-CH_3); MS (EI): *m/z* 312 (M^+).

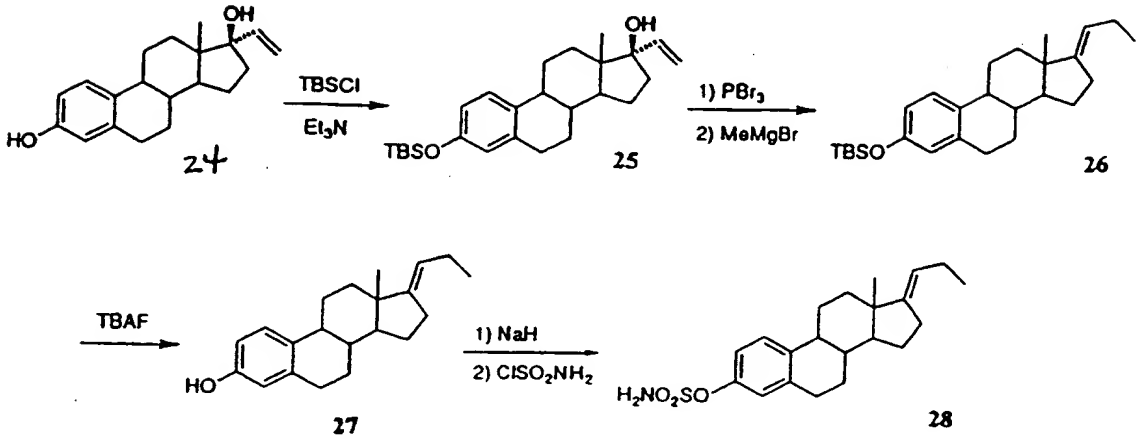
(c) Synthesis of 19,21-Dinorchola-1,3,5(10)-triene-3-*O*-sulfamate (**23**):

Beginning with 19,21-dinorchola-1,3,5(10)-trien-3-ol (**22**, 0.177 g, 0.57 mmol), by using the procedure described in step (b) of Example 4 above, there was obtained 0.198 g of **23** (89% yield; mp: 144-145°C) after chromatography (n-hexane:EtOAc 5:1°2:1, v/v).

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.13-6.97 (m, 2H, aromatic), 4.90 (s, 2H, -NH_2), 0.90 (t, 3H, 24-CH_3), 0.61 (s, 3H, 18-CH_3); MS (EI): *m/z* 391 (M^+).

Example 10

Preparation of 3-*tert*-Butyldimethylsilyloxy-17α-ethenylestra-1,3,5(10)-trien-17β-ol (28)



Scheme 4

(a) Synthesis of 3-*tert*-Butyldimethylsilyloxy-17α-ethenylestra-1,3,5(10)-trien-17β-ol (25):

To a solution of 17α-ethenylestradiol (**24**, 0.298 g, 1.0 mmol) in 1,2-dichloroethane (5.0 mL) and THF (1.0 mL) were added triethylamine (0.35 mL, 2.5 mmol) and *tert*-butyldimethylchlorosilane (0.226 g, 1.5 mmol) and 4-dimethylaminopyridine (0.006 g, 0.05 mmol) at room temperature. The reaction mixture was stirred for 2 days, diluted with EtOAc, washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1~3:1, v/v) to afford 0.358 g of **25** (87% yield) mp: 127-128°C.

^{1}H NMR: δ 7.10 (d, 1H, aromatic), 6.67-6.52 (m, 2H, aromatic), 6.12 (dd, 1H, -C<u>H</u>=CH_2), 5.25-5.13 (m, 2H, -CH=C<u>H</u>$_2$), 0.97 (s, 9H, -$C(CH_3)_3$), 0.95 (s, 3H, 18-CH_3), 0.18 (s, 6H, -$Si(CH_3)_2$).

(b) Synthesis of 3-*tert*-Butyldimethylsilyloxy-[17(20)E]-propylideneestra-1,3,5(10)-triene (**26**):

To a solution of phosphorous tribromide (4.5 mL of a CH_2Cl_2 solution, 1.0 M, 4.5 mmol) in toluene (6.0 mL) was added a solution of 3-*tert*-butyldimethylsilyloxy-17α-ethenylestra-1,3,5(10)-trien-17β-ol (**25**, 1.86 g, 4.5 mmol) and pyridine (0.40 mL, 5.0 mmol) in toluene (25 mL) at 0°C. The reaction mixture was stirred for 2 h, quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was dissolved in THF (20 mL) and added methylmagnesiumbromide (7.5 mL of a Et_2O solution, 3.0 M, 22.5 mmol) at 0°C. The reaction mixture was stirred for 19 h at room temperature, quenched with H_2O at 0°C and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The residue was purified by column chromatography (silica gel) using n-hexane:$CHCl_3$ (5:1~3:1, v/v) to afford 1.06 g of **26** (57% yield) mp: 59-60°C.

^{1}H NMR: δ 7.13 (d, 1H, aromatic), 6.66-6.51 (m, 2H, aromatic), 5.08-4.95 (m, 1H, =C<u>H</u>-CH_2-), 0.98 (s, 9H, -$C(CH_3)_3$), 0.93 (t, 3H, 23-CH_3), 0.78 (s, 3H, 18-CH_3), 0.18 (s, 6H, -$Si(CH_3)_2$).

(c) Synthesis of [17(20)E]-Propylideneestra- 1,3,5(10)-trien-3-ol (**27**):

To a solution of 3-*tert*-butyldimethylsilyloxy-[17(20)E]-propylideneestra-1,3,5(10)-triene (**26**, 0.821 g, 2.0 mmol) in THF (20 mL) was added tetrabutylammonium fluoride (2.4 mL of a THF solution, 1.0 M, 2.4 mmol) at 0°C. The reaction mixture was stirred for 1 h, diluted with EtOAc, washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (10:1~5:1, v/v) to afford 0.598 g of **27** (100% yield) mp: 105-106°C.

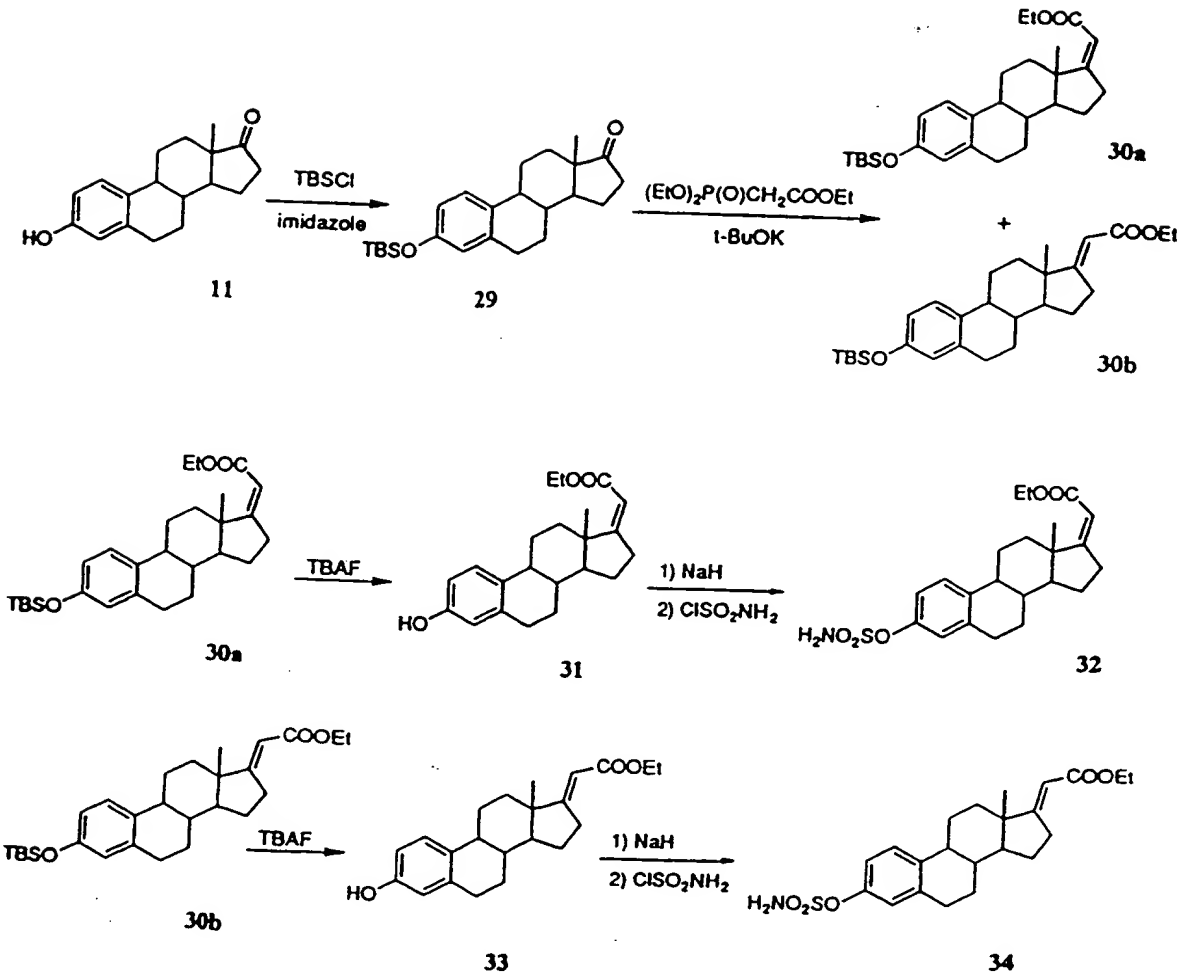
^{1}H NMR: δ 7.17 (d, 1H, aromatic), 6.67-6.52 (m, 2H, aromatic), 5.06-4.95 (m, 1H, =C<u>H</u>-CH_2-), 4.53 (s, 1H, -OH), 0.95 (t, 3H, 23-CH_3), 0.78 (s, 3H, 18-CH_3); MS (EI): *m/z* 296 (M^+).

(d) Synthesis of [17(20)E]-Propylideneestra-1,3,5(10)-triene-3-*O*-sulfamate (**28**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of [17(20)E]- propylideneestra-1,3,5(10)-trien-3-ol (**27**, 0.148 g, 0.5 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1→3:1, v/v) to afford 0.177 g of **28** (94% yield) mp: 149-151°C.

^{1}H NMR: δ 7.33 (d, 1H, aromatic), 7.13-7.00 (m, 2H, aromatic), 5.08-4.95 (m, 1H, =C<u>H</u>-CH_2-), 4.89 (s, 2H, -NH_2), 0.96 (t, 3H, 23-CH_3), 0.79 (s, 3H, 18-CH_3); MS (EI): *m/z* 375 (M^+).

The following scheme illustrates the synthetic steps carried out in Examples 11 and 12 to make the anti-estrogenic compounds (**32**) and (**34**):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TBSCl
imidazole
TBSO
11
29
(EtO)2P(O)CH2COOEt
t-BuOK
EtOOC
TBSO
30a
+
COOEt
TBSO
30b
TBSO
TBAF
HO
1) NaH
2) ClSO2NH2
H2NO2SO
30a
31
32
TBSO
TBAF
HO
1) NaH
2) ClSO2NH2
H2NO2SO
30b
33
34

Scheme 5

Example 11

Preparation of Ethyl 3-Sulfamoyloxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (32)

(a) Synthesis of 3-*tert*-Butyldimethylsilyloxyestra- 1,3,5(10)-trien-17-one (**29**):

To a solution of estrone (**11**, 8.10 g, 30.0 mmol) in DMF (25 mL) were added imidazole (3.07 g, 45 mmol) and *tert*-butyldimethyl-chlorosilane (5.42 g, 36 mmol) at room temperature. The reaction mixture was stirred for 18 h, and then quenched with H_2O (100 mL). The precipitate was collected by filtration and washed with H_2O to afford 11.4 g of **29** (99% yield) mp: 171-172°C.

^{1}H NMR: δ 7.12 (d, 1H, aromatic), 6.67-6.55 (m, 2H, aromatic), 0.98 (s, 9H, -C(CH_3)$_3$), 0.91 (s, 3H, 18-CH_3), 0.19 (s, 6H, -Si(CH_3)$_2$).

(b) Synthesis of Ethyl-3-*tert*-Butyldimethylsilyloxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**30a**) and Ethyl 3-*tert*-Butyldimethylsilyloxy-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**30b**):

To a solution of triethylphosphonoacetate (3.17 mL, 16 mmol) in THF (40 mL) was added potassium *tert*-butoxide (1.68 g, 15 mmol) at room temperature. The reaction mixture was stirred for 30 min, and 3-*tert*-butyldimethylsilyloxyestra-1,3,5(10)-trien-17-one (**29**, 1.92 g, 5.0 mmol) was added. The stirring continued for 2 days at reflux condition. After the reaction mixture was cooled to room temperature, saturated aqueous NH_4Cl was added and the mixture extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (40:1−30:1, v/v) to afford 0.473 g of **30a** (21% yield) mp: 148-149°C, and 1.26 g of **30b** (55% yield) mp: 109-110°C.

^{1}H NMR (**30a**): δ 7.12 (d, 1H, aromatic), 6.65-6.50 (m, 2H, aromatic), 5.70-5.63 (m, 1H, =C<u>H</u>-COOEt-), 4.23-4.05 (m, 2H, -COOC<u>H</u>$_2$CH$_3$), 1.29 (t, 3H, -COOCH$_2$C<u>H</u>$_3$), 1.04 (s, 3H, 18-CH_3), 0.98 (s, 9H, -C(CH_3)$_3$), 0.18 (s, 6H, -Si(CH_3)$_2$).

^{1}H NMR (**30b**): δ 7.12 (d, 1H, aromatic), 6.65-6.50 (m, 2H, aromatic), 5.59 (s, 1H, =C<u>H</u>-COOEt-), 4.16 (q, 2H, -COOC<u>H</u>$_2$CH$_3$), 1.29 (t, 3H, -COOCH$_2$C<u>H</u>$_3$), 0.98 (s, 9H, -C(CH$_3$)$_3$), 0.86 (s, 3H, 18-CH$_3$), 0.19 (s, 6H, Si(CH$_3$)$_2$).

(c) Synthesis of Ethyl 3-Hydroxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**31**):

To a solution of ethyl 3-*tert*-butyldimethylsilyloxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**30a**, 0.387 g, 0.85 mmol) in THF (10 mL) was added tetrabutylammonium fluoride (0.90 mL of a THF solution, 1.0 M, 0.90 mmol) at 0°C. The reaction mixture was stirred for 1 h, diluted with EtOAc, washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (7:1–5:1, v/v) to afford 0.279 g of **31** (97% yield) mp: 144-145°C.

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.67-6.52 (m, 2H, aromatic), 5.72-5.66 (m, 1H, =C<u>H</u>-COOEt), 4.23-4.08 (m, 2H, -COOC<u>H</u>$_2$CH$_3$), 1.29 (t, 3H, -COOCH$_2$C<u>H</u>$_3$), 1.04 (s, 3H, 18-CH$_3$); MS (EI): *m/z* 340 (M$^+$).

(d) Synthesis of Ethyl 3-Sulfamoyloxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**32**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of ethyl 3-hydroxy- [17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**31**, 0.170 g, 0.5 mmol) in DMF (3.0 mL) and THF (1.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1–2:1, v/v) to afford 0.174 g of **32** (83% yield) mp: 154-155°C.

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.15-7.00 (m, 2H, aromatic), 5.69 (s, 1H, =C<u>H</u>-COOEt), 4.93 (s, 1H, -NH_2), 4.15 (q, 2H, -COO<u>CH_2</u>CH_3), 1.29 (t, 3H, -$COOCH_2$<u>CH_3</u>), 1.04 (s, 3H, 18-CH_3); MS (EI): *m/z* 419 (M^+).

Example 12

Preparation of Ethyl 3-Sulfamoyloxy-[17(20)E]-19-norpregna-1,3,5(10),20-tetraen-21-oate (34)

(a) Synthesis of Ethyl 3-*tert*-Butyldimethyl-silyloxy-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**30b**):

The procedure described in steps (a) and (b) of Example 11 above was used to obtain **30b** from **11**.

(b) Synthesis of Ethyl 3-Hydroxy-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**33**):

By using the procedure described in step (c) of Example 11 above, beginning with ethyl 3-*tert*-butyldimethylsilyloxy-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**30b**, 1.00 g, 2.2 mmol), there was obtained 0.727 g of **33** (97% yield; mp: 153-154°C) after chromatography (n-hexane:EtOAc 5:1→3:1, v/v).

^{1}H NMR: δ 7.15 (d, 1H, aromatic), 6.68-6.53 (m, 2H, aromatic), 5.59 (s, 1H, =C<u>H</u>-COOEt-), 4.77-4.65 (m, 1H, -OH), 4.17 (q, 2H, -COO<u>CH_2</u>CH_3), 1.29 (t, 3H, -$COOCH_2$<u>CH_3</u>), 0.86 (s, 3H, 18-CH_3); MS (EI): *m/z* 340 (M^+).

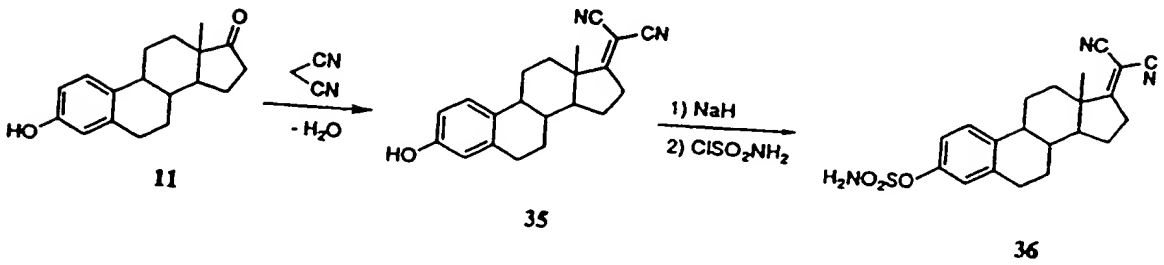
(c) Synthesis of Ethyl 3-Sulfamoyloxy-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**34**):

By the procedure described in step (d) of Example 11 above, there was obtained from ethyl 3-hydroxy- [17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-21-oate (**33**, 0.102 g, 0.3 mmol) after chromatography (n-hexane:EtOAc 5:1→3:1, v/v), 0.097 g of **34** (77% yield) mp: 174-175°C.

^{1}H NMR: δ 7.32 (d, 1H, aromatic), 7.13-7.00 (m, 2H, aromatic), 5.62-5.57 (m, 1H, =C<u>H</u>-COOEt), 4.89 (s, 1H, -NH_2), 4.16 (q, 2H, -COO<u>CH_2</u>CH_3), 1.29 (t, 3H, -$COOCH_2$<u>CH_3</u>), 0.87 (s, 3H, 18-CH_3); MS (EI): *m/z* 419 (M^+).

Example 13

Preparation of 20-Cyano-19-Norpregna-1,3,5(10),17(20)-tetraene-21-nitrile-3-*O*-sulfamate (36)



11 35 36

Scheme 6

(a) Synthesis of 20-Cyano-3-hydroxy-19-norpregna-1,3,5(10),17(20)-tetraene-21-nitrile (35):

To a suspension of estrone (11, 1.35 g, 5.0 mmol) in benzene (35 mL) and acetic acid (5.0 mL) were added malononitrile (1.65 g, 40 mmol) and β-alanine (0.535 g, 6.0 mmol), and stirred for 19 h at reflux condition. After the reaction mixture was cooled to room temperature, H_2O was added and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 1.46 g of 35 (92% yield) mp: >250°C.

^{1}H NMR: δ 7.13 (d, 1H, aromatic), 6.70-6.53 (m, 2H, aromatic), 1.07 (s, 3H, 18-CH_3); MS (EI): *m/z* 318 (M$^+$).

(b) Synthesis of 20-Cyano-19-norpregna-1,3,5(10),17(20)-tetraene-21-nitrile-3-*O*-sulfamate (36):

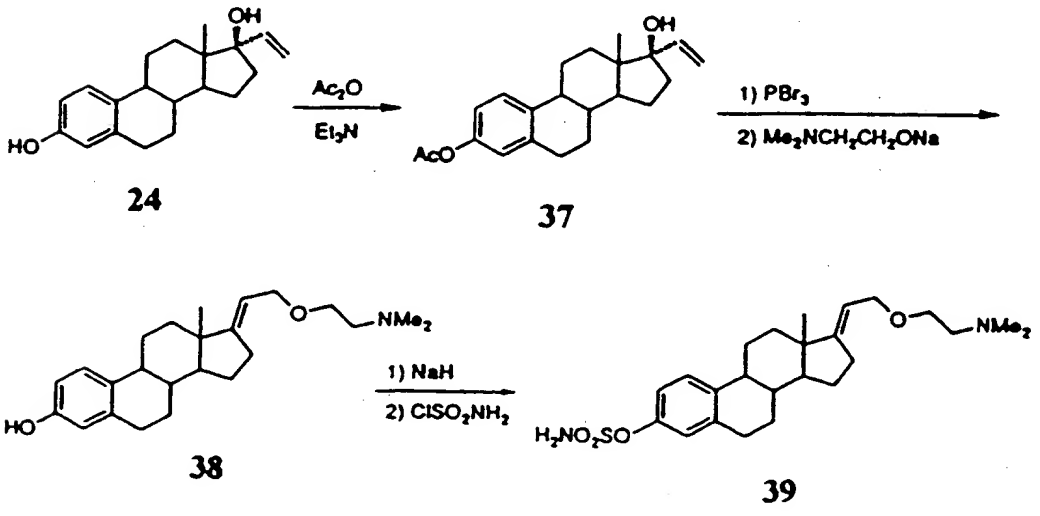
To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at

0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 20-cyano-3-hydroxy-19-norpregna-1,3,5(10),17(20)-tetraene-21-nitrile (**35**, 0.159 g, 0.5 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:acetone (3:1→3:2, v/v) to afford 0.152 g of **36** (77% yield) mp: 183-184°C.

^{1}H NMR: δ 7.31 (d, 1H, aromatic), 7.20-7.00 (m, 2H, aromatic), 4.93 (s, 1H, $-NH_2$), 1.08 (s, 3H, 18-CH_3); MS (EI): *m/z* 397 (M^+).

Example 14

Preparation of 21-(2'-*N,N*-Dimethylaminoethoxy)-[17(20)E]-19-norpregna-1,3,5(10),18(20)-tetraene-3-*O*-sulfamate (**39**)



24 **37** **38** **39**

Scheme 7

(a) Synthesis of 17α-Ethenylestra-1,3,5(10)-trien-3,17β-diol 3-*O*-acetate (**37**):

To a solution of 17α-ethenylestradiol (**24**, 0.895 g, 3.0 mmol) in CH_2Cl_2 (7.0 mL) and THF (3.0 mL) were added triethylamine (0.95 mL, 6.7 mmol) and acetic anhydride (0.4 mL, 4.0 mmol) at room temperature. The reaction mixture was stirred for 20 h, diluted with EtOAc, washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1→2:1, v/v) to afford 1.02 g of **37** (100% yield) mp: 127-128°C.

^{1}H NMR: δ 7.26 (d, 1H, aromatic), 6.88-6.77 (m, 2H, aromatic), 6.10 (dd, 1H, -C<u>H</u>=CH_2), 5.25-5.10 (m, 2H, -CH=C<u>H</u>$_2$), 2.27 (s, 3H, -$OCOCH_3$), 0.94 (s, 3H, 18-CH_3).

(b) Synthesis of 21-(2'-*N*,*N*-Dimethylaminoethoxy)-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**38**):

To a solution of phosphorous tribromide (3.2 mL of a CH_2Cl_2 solution, 1.0 M, 3.2 mmol) in toluene (4.0 mL) was added a solution of 17α-ethenylestra-1,3,5(10)-trien-3,17β-diol 3-*O*-acetate (**37**, 1.09 g, 3.2 mmol) and pyridine (0.3 mL, 3.7 mmol) in toluene (20 mL) at 0°C. The reaction mixture was stirred for 2 h, and quenched with saturated aqueous NH_4Cl at 0°C and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was dissolved in THF (20 mL) and added to a mixture of *N*,*N*-dimethylethanolamine (3.0 mL, 30 mmol) and sodium hydride (1.00 g of a mineral oil dispersion, 60%, 25 mmol) in THF (20 mL) at 0°C. The reaction mixture was stirred for 2 h, and quenched with saturated aqueous $NaHCO_3$ at 0°C and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (10:1→7:1, v/v) to afford 0.309 g of **38** (26% yield) mp: 116-117°C.

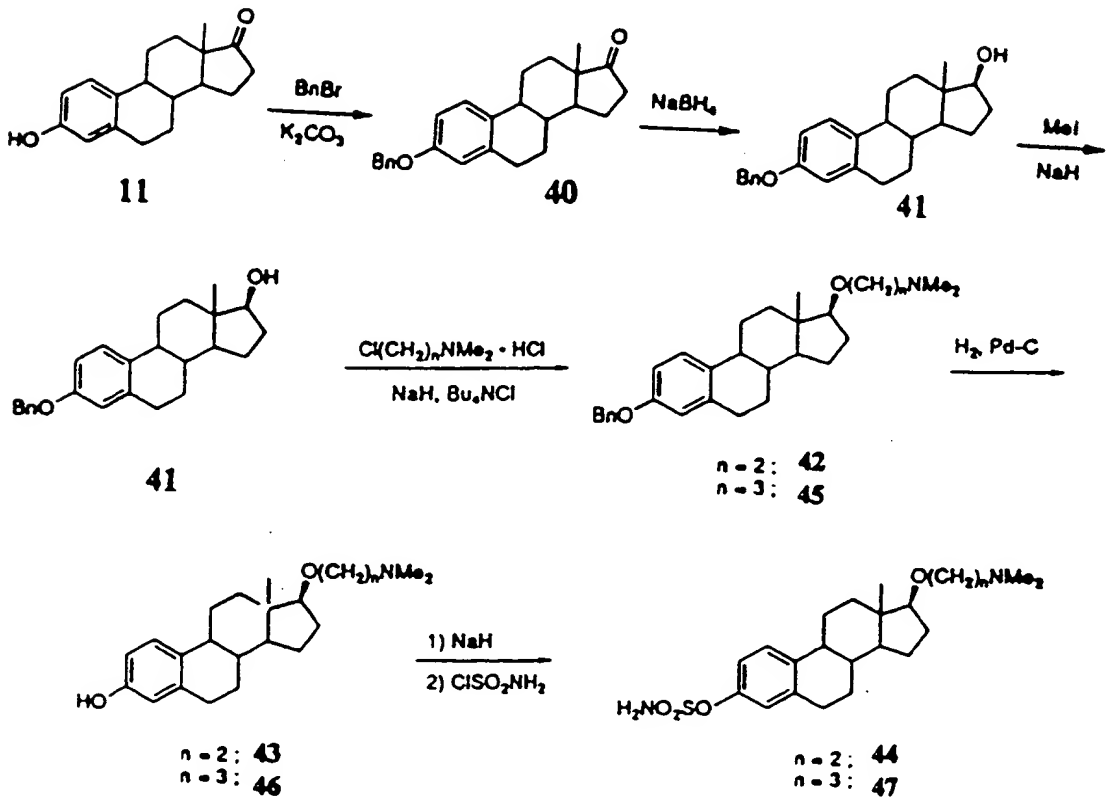
^{1}H NMR: δ 7.12 (d, 1H, aromatic), 6.63-6.48 (m, 2H, aromatic), 5.25-5.14 (m, 1H, =C<u>H</u>-CH_2O-), 3.98 (d, 2H, =CH-C<u>H_2</u>O-), 3.65-3.45 (m, 2H, -OC<u>H_2</u>CH_2N-), 2.61 (t, 2H, -OCH_2C<u>H_2</u>N-), 2.34 (s, 6H, -N(CH_3)$_2$), 0.76 (s, 3H, 18-CH_3); MS (DCI): *m/z* 370 (M^+H).

(c) Synthesis of 21-(2'-*N*,*N*-Dimethylaminoethoxy)- [17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraene- 3-*O*-sulfamate (**39**):

To a solution of chlorosulfonyl isocyanate (0.14 mL, 1.5 mmol) in CH_2Cl_2 (0.6 mL) was added formic acid (0.3 mL of a CH_2Cl_2 solution, 5.0 M, 1.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 21-(2'-*N*,*N*-dimethylaminoethoxy)-[17(20)E]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**38**, 0.111 g, 0.3 mmol) in DMF (2.0 mL) was added sodium hydride (0.060 g of a mineral oil dispersion, 60%, 1.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (10:1→5:1, v/v) to afford 0.121 g of **39** (90% yield) mp: 147-148°C.

^{1}H NMR: δ 7.30 (d, 1H, aromatic), 7.13-7.00 (m, 2H, aromatic), 5.30-5.18 (m, 1H, =C<u>H</u>-CH_2O-), 4.05-3.90 (m, 2H, =CH-C<u>H_2</u>O-), 3.53 (t, 2H, -OC<u>H_2</u>CH_2N-), 2.55 (t, 2H, -OCH_2C<u>H_2</u>N-), 2.30 (s, 6H, -N(CH_3)$_2$), 0.78 (s, 3H, 18-CH_3); MS (DCI): *m/z* 449 (M^++H).

The following scheme illustrates the synthetic steps carried out in Examples 15 and 16 to make the estrone sulfatase inhibitory compounds (44) and (47):



Scheme 8

Example 15

Preparation of 17β-(2'-*N*,*N*-Dimethylaminoethoxy)estra-1,3,5(10)-triene-3-*O*-sulfamate (44)

(a) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17-one (40):

To a solution of estrone (11, 2.70 g, 10 mmol) in DMF (40 mL) were added potassium carbonate (2.76 g, 20 mmol) and benzyl bromide (1.8 mL, 15 mmol) at room temperature. The reaction mixture was stirred for 26 h, then quenched with H_2O, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 2.91 g of **40** (81% yield) mp: 126-127°C.

^{1}H NMR: δ 7.65-7.10 (m, 6H, aromatic), 6.90-6.65 (m, 2H, aromatic), 5.04 (s, 2H, -OCH_2Ph), 0.91 (s, 3H, 18-CH_3); MS (EI): *m/z* 360 (M^+).

(b) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17β-ol (41):

To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17-one (**40**, 2.70 g, 7.5 mmol) in THF (5.0 mL) and MeOH (30 mL) was added sodium borohydride (284 mg, 7.5 mmol) at 0°C. The reaction mixture was stirred for 30 min, then quenched with saturated aqueous NH_4Cl, and H_2O added. The precipitate was collected by filtration and washed with H_2O to afford 2.73 g of **41** (100% yield) mp: 118-119°C.

^{1}H NMR: δ 7.50-7.15 (m, 6H, aromatic), 6.83-6.67 (m, 2H, aromatic), 5.03 (s, 2H, -OCH_2Ph), 3.80-3.65 (m, 2H, 17*a*-H, -OH), 0.78 (s, 3H, 18-CH_3); MS (EI): *m/z* 362 (M^+).

(c) Synthesis of 3-Benzyloxy-17β-(2'-*N*,*N*-dimethyl-aminoethoxy)estra-1,3,5(10)-triene (42):

To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17β-ol (**41**, 1.81 g, 5.0 mmol) in DMF (40 mL) was added sodium hydride (3.00 g of a mineral oil dispersion, 60%, 75 mmol) at 0°C, and stirred for 30 min, then added 2-*N*,*N*-dimethylaminoethylchloride hydrochloride (2.16 g, 15 mmol) and tetrabutylammonium iodide (0.185 g, 0.50 mmol) and stirred for 3 h at 100°C. After the reaction mixture was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the mixture extracted with EtOAc. The combined organic layers were

washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (30:1→10:1, v/v) to afford 1.82 g of **42** (84% yield) mp: 153-155°C.

^{1}H NMR: δ 7.50-7.15 (m, 6H, aromatic), 6.85-6.67 (m, 2H, aromatic), 5.03 (s, 2H, -OCH_2Ph), 4.03-3.85 (m, 2H, 17β-OCH_2-), 3.44 (t, 1H, 17α-H), 2.89 (s, 6H, -$N(CH_3)_2$), 0.77 (s, 3H, 18-CH_3).

(d) Synthesis of 17β-(2'-*N*,*N*-Dimethylamino-ethoxy)estra-1,3,5(10)-trien-3-ol (**43**):

To a solution of 3-benzyloxy-17β-(2'-*N*,*N*-dimethylaminoethoxy) estra-1,3,5(10)-triene (**42**, 1.73 g, 4.0 mmol) in MeOH (20 mL) was added 10% palladium on carbon (0.500 g). The reaction mixture was stirred for 2 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (10:1→3:1, v/v) to afford 1.27 g of **43** (92% yield) mp: 191-192°C.

^{1}H NMR: δ 7.13 (d, 1H, aromatic), 6.68-6.48 (m, 2H, aromatic), 3.73-3.48 (m, 2H, 17β-OCH_2-), 3.37 (t, 1H, 17α-H), 2.33 (s, 6H, -$N(CH_3)_2$), 0.74 (s, 3H, 18-CH_3); MS (DCI): *m/z* 344 (M^++H).

(e) Synthesis of 17β-(2'-*N*,*N*-Dimethylaminoethoxy)estra-1,3,5(10)-triene-3-*O*-sulfamate (**44**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 17β-(2'-*N*,*N*-dimethylaminoethoxy)estra-1,3,5(10)-trien-3-ol (**43**, 0.172 g, 0.5 mmol) in DMF (3.0 mL) and THF (1.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant

was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (10:1°5:1, v/v) to afford 0.180 g of **44** (85% yield) mp: 142-143°C.

^{1}H NMR: δ 7.28 (d, 1H, aromatic), 7.13-7.00 (m, 2H, aromatic), 3.90-3.45 (m, 4H, 17β-OCH_2-, -NH_2), 3.37 (t, 1H, 17α-H), 2.34 (s, 6H, -$N(CH_3)_2$), 0.73 (s, 3H, 18-CH_3); MS (DCI): *m/z* 423 (M^++H).

Example 16

Preparation of 17β-(3'-*N,N*-Dimethylaminopropoxy)estra-1,3,5(10)-triene-3-*O*-sulfamate (47)

(a) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17β-ol (**41**):

The procedure described in steps (a) and (b) of Example 15 above was used to obtain **41**.

(b) Synthesis of 3-Benzyloxy-17(3-*N,N*-dimethylaminopropoxy)estra-1,3,5(10)-triene (**45**):

To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17β-ol (**41**, 1.27 g, 3.5 mmol) in DMF (30 mL) were added sodium hydride (2.10 g of a mineral oil dispersion, 60%, 52.5 mmol) at 0°C, and stirred for 30 min. Next was added 3-*N,N*-dimethylaminopropylchloride hydrochloride (1.66 g, 10.5 mmol) and tetrabutylammonium iodide (0.129 g, 0.35 mmol) and stirred for 19 h at 100°C. After the reaction mixture was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the mixture extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (20:1→10:1, v/v) to afford 1.57 g of **45** (100% yield) mp: 190-192°C.

^{1}H NMR: δ 7.50-7.15 (m, 6H, aromatic), 6.83-6.67 (m, 2H, aromatic), 5.03 (s, 2H, -OCH_2Ph), 3.60-3.43 (m, 2H, 17β-OCH_2-), 3.37 (t, 1H, 17α-H), 2.28 (s, 6H, -$N(CH_3)_2$), 0.78 (s, 3H, 18-CH_3).

(c) Synthesis of 17β-(3'-*N,N*-Dimethylaminopropoxy)estra-1,3,5(10)-trien-3-ol (**46**):

By the procedure described in step (d) of Example 15 above, there was obtained from 3-benzyloxy-17β-(3'-*N,N*-dimethylaminopropoxy)estra-1,3,5(10)-triene (**45**, 1.57 g, 3.50 mmol) after washing with Et_2O, 0.992 g of **46** (79% yield) mp: >250°C.

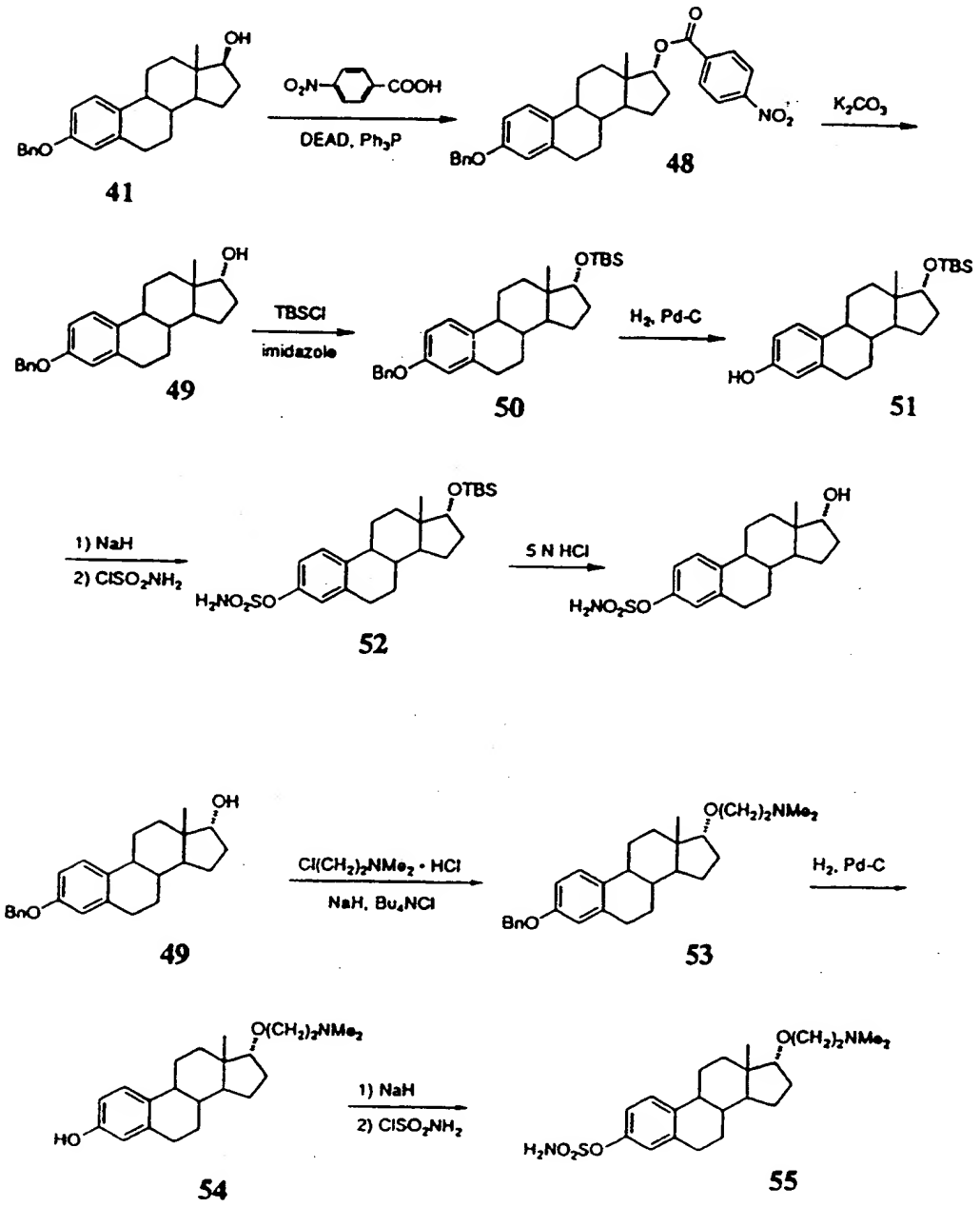
^{1}H NMR ($CDCl_3$-DMSO-d_6): δ 8.20 (s, 1H, -OH), 6.91 (d, 1H, aromatic), 6.50-6.33 (m, 2H, aromatic), 3.47-3.30 (m, 2H, 17β-OCH_2-), 3.18 (t, 1H, 17α-H), 2.64, 2.63 (s and s, each 3H, -$N(CH_3)_2$), 0.55 (s, 3H, 18-CH_3); MS (DCI): *m/z* 358 (M^++H).

(d) Synthesis of 17β-(3'-*N,N*-Dimethylaminopropoxy)estra-1,3,5(10)-triene-3-*O*-sulfamate (**47**):

By the procedure described in step (e) of Example 15 above, there was obtained from 17β-(3'-*N,N*-dimethylaminopropoxy)estra-1,3,5(10)-trien-3-ol (**46**, 0.179 g, 0.50 mmol) after chromatography ($CHCl_3$:MeOH 10:1–5:1, v/v), 0.161 g of **47** (74% yield) mp: 122-123°C.

^{1}H NMR ($CDCl_3$-DMSO-d_6): d 7.28 (d, 1H, aromatic), 7.11-7.00 (m, 2H, aromatic), 3.62-3.45 (m, 2H, 17β-OCH_2-), 3.38 (t, 1H, 17α-H), 2.41 (s, 6H, -$N(CH_3)_2$), 0.77 (s, 3H, 18-CH_3); MS (DCI): *m/z* 437 (M^++H).

The following scheme illustrates the synthetic steps carried out in Examples 17 and 18 to make the estrone sulfatase inhibitory compounds (52) and (55):



Scheme 9

Example 17

Preparation of 17*α-tert*-Butyldimethylsilyloxyestra-1,3,5(10)-triene-3-*O*-sulfamate (52)

(a) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17β-ol (**41**):

The procedure described in steps (a) and (b) of Example 15 above was used to obtain **41** from **11**.

(b) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17α-ol 17α-*O*-p-nitrobenzoate (**48**):

To a suspension of triphenylphosphine (6.29 g, 24 mmol) and diethyl azodicarboxylate (4.18 g 24 mmol) in toluene (40 mL) was added a solution of 3-benzyloxyestra-1,3,5(10)-trien-17*β*-ol (**41**, 4.35 g, 12.0 mmol) in toluene (40 mL) at room temperature, and stirred for 2 h at 80°C. After the reaction mixture was cooled to room temperature, H_2O was added and the mixture extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (10:1→7:1, v/v) to afford 5.82 g of **48** (95% yield) mp: 135-136°C.

^{1}H NMR: δ 8.37-8.18 (m, 4H, aromatic), 7.52-7.30 (m, 5H, aromatic), 7.19 (d, 1H, aromatic), 6.85-6.68 (m, 2H, aromatic), 5.15 (d, 1H, 17*β*-H), 5.03 (s, 2H, -OCH_2Ph), 0.88 (s, 3H, 18-CH_3).

(c) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17*α*-ol (**49**):

To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17*α*-ol 17*α*-*O*-p-nitrobenzoate (**48**, 6.14 g, 12 mmol) in THF (40 mL) and MeOH (40 mL) was added potassium carbonate (1.66 g, 12 mmol) and stirred for 2 h at room temperature. The reaction mixture was quenched with H_2O, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1→3:1, v/v) to afford 4.10 g of **49** (94% yield) mp: 85-86°C.

^{1}H NMR: δ 7.55-7.30 (m, 5H, aromatic), 7.22 (d, 1H, aromatic), 6.85-6.67 (m, 2H, aromatic), 5.03 (s, 2H, -OCH_2Ph), 3.81 (d, 1H, 17β-H), 0.70 (s, 3H, 18-CH_3).

(d) Synthesis of 3-Benzyloxy-17α-*tert*-butyldimethylsilyloxyestra-1,3,5(10)-triene (**50**):

To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17α-ol (**49**, 1.45 g, 4.0 mmol) in DMF (5.0 mL) were added imidazole (0.408 g, 6.0 mmol) and *tert*-butyldimethylchlorosilane (0.784 g, 5.2 mmol) at room temperature. The reaction mixture was stirred for 2 h, and quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:$CHCl_3$:EtOAc (50:50:1→20:20:1, v/v) to afford 1.91 g of **50** (100% yield).

^{1}H NMR: δ 7.53-7.25 (m, 5H, aromatic), 7.22 (d, 1H, aromatic), 6.85-6.68 (m, 2H, aromatic), 5.03 (s, 2H, -OCH_2Ph), 3.72 (d, 1H, 17β-H), 0.90 (s, 9H, -$C(CH_3)_3$), 0.66 (s, 3H, 18-CH_3), 0.04 (s, 6H, -$Si(CH_3)_2$).

(e) Synthesis of 17α-*tert*-butyldimethylsilyloxyestra-1,3,5(10)-trien-3-ol (**51**):

To a solution of 3-benzyloxy-17α-*tert*-butyldimethylsilyloxyestra-1,3,5(10)-triene (**50**, 1.90 g, 4.0 mmol) in THF (30 mL) was added 10% palladium on carbon (0.500 g). The reaction mixture was stirred for 2 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (10:1→5:1, v/v) to afford 1.45 g of **51** (94% yield) mp: 161-162°C.

^{1}H NMR: δ 7.16 (d, 1H, aromatic), 6.67-6.48 (m, 2H, aromatic), 4.53 (s, 1H, -OH), 3.71 (d, 1H, 17β-H), 0.90 (s, 9H, -$C(CH_3)_3$), 0.66 (s, 3H, 18-CH_3), 0.04 (s, 6H, -$Si(CH_3)_2$); MS (EI): *m/z* 386 (M^+).

(f) Synthesis of 17α-*tert*-Butyldimethylsilyloxyestra-1,3,5(10)-triene-3-*O*-sulfamate (**52**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 17α-*tert*-butyldimethylsilyloxyestra-1,3,5(10)-trien-3-ol (**51**, 0.193 g, 0.5 mmol) in DMF (3.0 mL) and THF (1.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1→3:1, v/v) to afford 0.216 g of **52** (93% yield) mp: 150-151°C.

^{1}H NMR: δ 7.33 (d, 1H, aromatic), 7.13-6.98 (m, 2H, aromatic), 4.87 (s, 2H, $-NH_2$), 3.72 (d, 1H, 17β-H), 0.90 (s, 9H, $-C(CH_3)_3$), 0.66 (s, 3H, 18-CH_3), 0.04 (s, 6H, $-Si(CH_3)_2$); MS (EI): *m/z* 465 (M^+).

Example 18

Preparation of 17α-(2'-*N,N*-Dimethylaminoethoxy)estra-1,3,5(10)-triene-3-*O*-sulfamate (55)

(a) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17α-ol 17α-*O*-p-nitrobenzoate (**48**):

The procedure described in steps (a) and (b) of Example 15 above was used to obtain **41** from **11**.

(b) Synthesis of 3-Benzyloxyestra-1,3,5(10)-trien-17α-ol (**49**):

The procedure described in steps (b) and (c) of Example 17 above was used to obtain **49** from **41**.

(c) Synthesis of 3-Benzyloxy-17α-(2'-*N,N*-dimethylaminoethoxy)estra-1,3,5(10)-triene (**53**):

To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17α-ol (**49**, 1.45 g, 4.0 mmol) in DMF (30 mL) were added sodium hydride (2.40 g of a mineral oil

dispersion, 60%, 60 mmol) at 0°C, and stirred for 30 min, added 2-*N*,*N*-dimethyl-aminoethylchloride hydrochloride (1.73 g, 12 mmol) and tetrabutylammonium iodide (0.148 g, 0.40 mmol) and stirred for 2 h at 100°C. After the reaction mixture was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the mixture extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (30:1–15:1, v/v) to afford 1.56 g of **53** (90% yield) mp: 190-191°C.

^{1}H NMR: δ 7.53-7.16 (m, 6H, aromatic), 6.83-6.67 (m, 2H, aromatic), 5.03 (s, 2H, -OCH_2Ph), 4.05-3.70 (m, 2H, 17α-OCH_2-), 3.42 (d, 1H, 17β-H), 2.88 (s, 6H, -$N(CH_3)_2$), 0.72 (s, 3H, 18-CH_3).

(d) Synthesis of 17α-(2'-*N*,*N*-Dimethylaminoethoxy)estra-1,3,5(10)-trien-3-ol (**54**):

To a solution of 3-benzyloxy-17α-(2'-*N*,*N*-dimethylaminoethoxy)estra-1,3,5(10)-triene (**53**, 1.52 g, 3.5 mmol) in MeOH (20 mL) was added 10% palladium on carbon (0.500 g). The reaction mixture was stirred for 2 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 1.02 g of **76** (85% yield) mp: 233-235°C.

^{1}H NMR ($CDCl_3$-DMSO-d_6): δ 7.10 (d, 1H, aromatic), 6.72-6.55 (m, 2H, aromatic), 4.05-3.70 (m, 2H, 17α-OCH_2-), 3.41 (d, 1H, 17β-H), 2.86 (s, 6H, -$N(CH_3)_2$), 0.71 (s, 3H, 18-CH_3); MS (DCI): *m/z* 344 (M^++H).

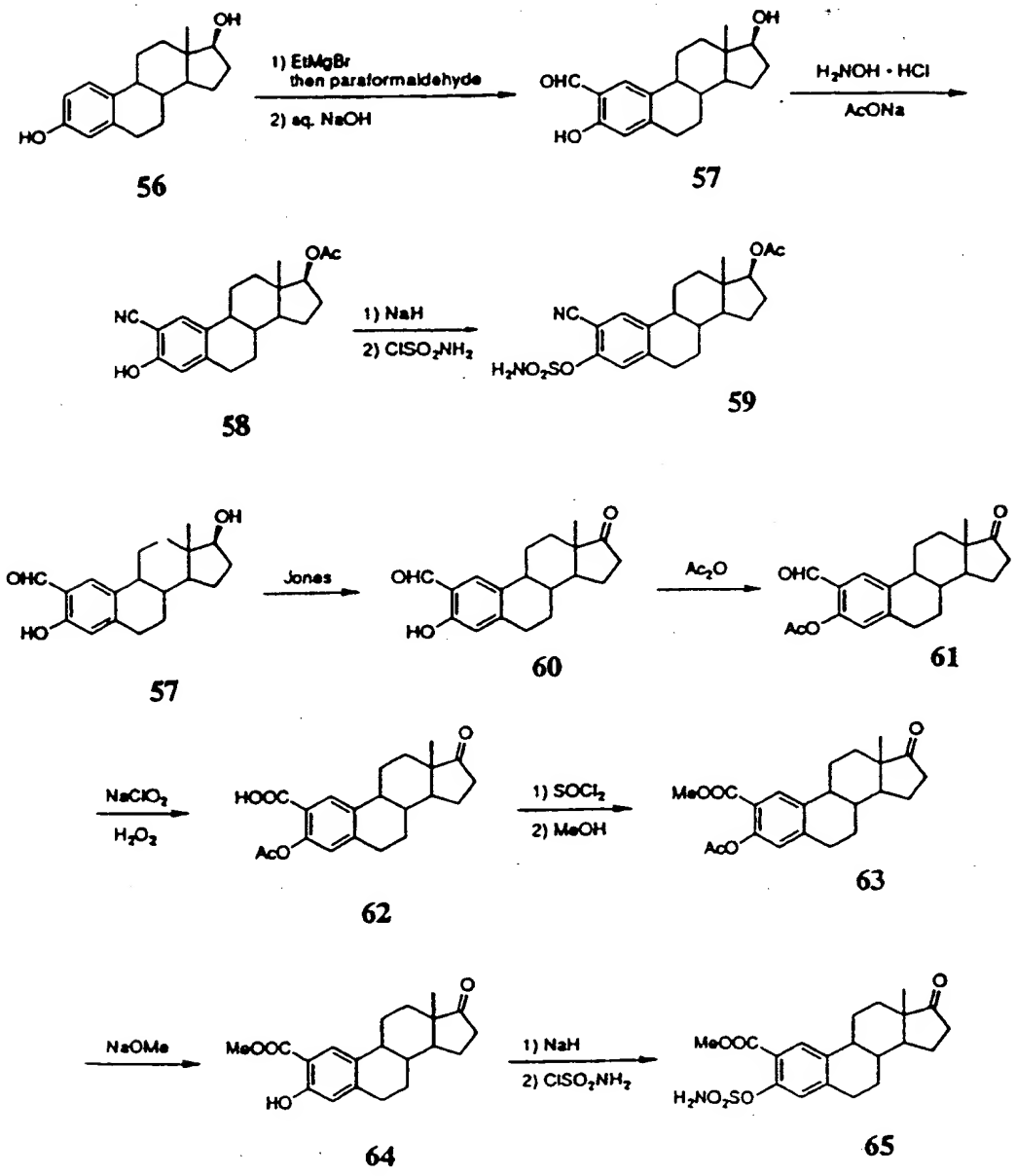
(e) Synthesis of 17α-(2'-*N*,*N*-Dimethylaminoethoxy)estra-1,3,5(10)-triene-3-*O*-sulfamate (**55**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 17α-(2'-*N*,*N*-dimethylaminoethoxy)estra-1,3,5(10)-trien-3-ol (**54**, 0.172 g, 0.5 mmol) in DMF (3.0 mL) and THF (1.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was

stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 4 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (12:1→7:1, v/v) to afford 0.116 g of **55** (55% yield) mp: 136-138°C.

^{1}H NMR: δ 7.27 (d, 1H, aromatic), 7.15-6.98 (m, 2H, aromatic), 6.20-5.65 (m, 2H, $-NH_2$), 3.68-3.37 (m, 2H, 17α-OCH_2-), 3.31 (d, 1H, 17β-H), 2.33 (s, 6H, $-N(CH_3)_2$), 0.67 (s, 3H, 18-CH_3); MS (DCI): *m/z* 423 (M^++H).

The following scheme illustrates the synthetic steps carried out in Examples 19 and 20 to make the estrone sulfatase inhibitory compounds (59) and (65):



Scheme 10

Example 19

Preparation of 2-Cyanoestra-1,3,5(10)-trien-17β-ol-3-*O*-sulfamate 17β-*O*-acetate (59)

(a) Synthesis of 3,17β-Dihydroxyestra-1,3,5(10)-triene-2-carboxaldehyde (57):

To a suspension of magnesium (2.07 g, 85 mmol) in THF (20 mL) was added bromoethane (8.9 mL, 119 mmol) dissolved in THF (15 mL) at room temperature. Estradiol (56, 4.63 g, 17 mmol) dissolved in THF (40 mL) was added to the reaction mixture, and stirring continued for 30 min. The solvent was removed at reduced pressure, and to the residue were added benzene (200 mL), hexamethylphosphoric triamide (7.4 mL, 42.5 mmol) and paraformaldehyde (7.00 g). Stirring was continued for 20 h at 80°C. After the reaction mixture was cooled to room temperature, 5 N HCl (150 mL) was added and the mixture extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was dissolved in MeOH (200 mL), 20% aqueous sodium hydroxide (25 mL) added, and the mixture stirred for 30 min at room temperature. The reaction mixture was acidified with 5 N HCl at 0°C, the solvent evaporated at reduced pressure, and the residue extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (N_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:THF (5:1→2:1, v/v) to afford 4.81 g of 57 (94% yield) mp: 219-221°C.

^{1}H NMR: δ 10.77 (s, 1H, -OH), 9.81 (s, 1H, -CHO), 7.42 (s, 1H, aromatic), 6.70 (s, 1H, aromatic), 3.74 (t, 1H, 17α-H), 0.79 (s, 3H, 18-CH_3); MS (EI): *m/z* 300 (M^+).

(b) Synthesis of 2-Cyanoestra-1,3,5(10)-trien-3,17-β-diol 17β-*O*-acetate (58):

To a suspension of 3,17β-dihydroxyestra-1,3,5(10)-triene-2-carboxaldehyde (57, 0.300 g, 1.0 mmol) in acetic acid (6.0 mL) was added sodium acetate (1.23 g, 15 mmol), hydroxylamine hydrochloride (0.139 g, 2.0 mmol). The reaction mixture was

stirred for 18 h under the reflux condition. After the reaction mixture was cooled to room temperature, diluted with EtOAc, and washed with H_2O, saturated aqueous NaCl, and then dried ($MgSO_4$). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1~2:1, v/v) to afford 0.259 g of **58** (76% yield) mp: 249-251°C.

^{1}H NMR: δ 7.38 (s, 1H, aromatic), 6.68 (s, 1H, aromatic), 4.69 (t, 1H, 17α-H), 2.07 (s, 3H, -$OCOCH_3$), 0.83 (s, 3H, 18-CH_3); MS (EI): *m/z* 339 (M^+); IR (nujol): 2229 cm^{-1}, 1733 cm^{-1}.

(c) Synthesis of 2-Cyanoestra-1,3,5(10)-trien-17β-ol-3-*O*-sulfamate 17β-*O*-acetate (**59**):

To a solution of chlorosulfonyl isocyanate (3.0 mL, 35 mmol) in CH_2Cl_2 (14 mL) was added formic acid (7.0 mL of a CH_2Cl_2 solution, 5.0 M, 35 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-cyanoestra-1,3,5(10)-trien-3-ol-17β-*O*-acetate (**58**, 2.38 g, 7.0 mmol) in DMF (40 mL) was added sodium hydride (1.40 g of a mineral oil dispersion, 60%, 35 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 5 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:EtOAc (5:1~2:1, v/v) to afford 0.676 g of the starting material **58** (29% yield) and 1.39 g of **59** (47% yield) mp: 182-183°C.

^{1}H NMR: d 7.56 (s, 1H, aromatic), 7.25 (s, 1H, aromatic), 5.43 (s, 2H, -NH_2), 4.70 (t, 1H, 17α-H), 2.07 (s, 3H, -$OCOCH_3$), 30.83 (s, 3H, 18-CH_3); MS (EI): *m/z* 418 (M^+); HRMS calcd for $C_{21}H_{25}N_2O_5S_1$ 417.1484, found 417.1476; IR (nujol): 3319 cm^{-1}, 3216 cm^{-1}, 2233 cm^{-1}, 1703 cm^{-1}.

Example 20

Preparation of 2-Methoxycarbonylestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (65)

(a) Synthesis of 3,17β-Dihydroxyestra-1,3,5(10)-triene-2-carboxaldehyde (57):

The procedure described in step (a) of Example 19 above was used to obtain **57** from **56**.

(b) Synthesis of 3-Hydroxyestra-1,3,5(10)-trien-17-one-2-carboxaldehyde (**60**):

To a solution of 3,17β-dihydroxyestra-1,3,5(10)-triene-2-carboxaldehyde (**57**, 0.300 g, 1.0 mmol) in acetone (20 mL) was added Jones reagent (0.5 mL) at 0°C. The reaction mixture was stirred for 5 min, and quenched with 2-propanol, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried ($MgSO_4$). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:THF (4:1→3:1, v/v) to afford 0.185 g of **60** (62 % yield) mp: 154-157°C.

^{1}H NMR: d 10.83 (s, 1H, -OH), 9.86 (s, 1H, -CHO), 7.47 (s, 1H, aromatic), 6.77 (s, 1H, aromatic), 0.97 (s, 3H, 18-CH_3); MS (EI): *m/z* 298 (M^+).

(c) Synthesis of 3-Acetoxyestra-1,3,5(10)-trien-17-one-2-carboxaldehyde (**61**):

To a solution of 3-hydroxyestra-1,3,5(10)-trien-17-one-2-carboxaldehyde (**60**, 1.92 g, 6.43 mmol) of CH_2Cl_2 (30 mL) was added triethylamine (2.3 mL, 16 mmol) and acetic anhydride (0.94 mL, 9.6 mmol) at room temperature. The reaction mixture was stirred for 16 h, H_2O was added, and the mixture was extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (2:1→3:2, v/v) to afford 2.08 g of **61** (95% yield) mp: 179-181°C.

^{1}H NMR: d 10.06 (s, 1H, -CHO), 7.83 (s, 1H, aromatic), 6.94 (s, 1H, aromatic), 2.42 (s, 3H, -$OCOCH_3$), 0.96 (s, 3H, 18-CH_3).

(d) Synthesis of 3-Acetoxyestra-1,3,5(10)-trien-17-one-2-carboxylic acid (**62**):

To a suspension of 3-acetoxyestra-1,3,5(10)-trien-17-one-2-carboxaldehyde (**61**, 1.20 g, 3.5 mmol) in acetonitrile (17 mL) and H_2O (2.1 mL) were added 30% hydrogen peroxide (0.53 mL) and sodium phosphate monobasic (1.79 g) at room temperature. Sodium chlorite (0.935 g in a H_2O (7.0 mL) solution) was added dropwise to the reaction mixture over a 1 h period, and stirring continued for an additional 2 h at room temperature. The reaction mixture was quenched with sodium sulfite, acidified with 10% HCl, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 1.06 g of **62** (85% yield) mp: 180-181°C.

^{1}H NMR: d 8.04 (s, 1H, aromatic), 6.86 (s, 1H, aromatic), 2.33 (s, 3H, -$OCOCH_3$), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 356 (M^+).

(e) Synthesis of Methyl 3-Acetoxyestra-1,3,5(10)-trien-17-one-2-carboxylate (**63**):

To a solution of 3-acetoxyestra-1,3,5(10)-trien-17-one-2-carboxylic acid (**62**, 1.07 g, 3.0 mmol) in dichloroethane (10 mL) was added thionyl chloride (0.28 mL, 3.9 mmol) and DMF (1 drop) at room temperature. The reaction mixture was stirred for 30 min at 80°C. After the reaction mixture was cooled to 0°C, MeOH (5.0 mL) and triethylamine (1.0 mL) were added, and stirred for 1 h. The reaction mixture was diluted with EtOAc and washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1→3:2, v/v) to afford 0.764 g of **63** (69% yield) mp: 182-183°C.

^{1}H NMR: d 7.95 (s, 1H, aromatic), 6.83 (s, 1H, aromatic), 3.85 (s, 3H, -$COOCH_3$), 2.34 (s, 3H, -$OCOCH_3$), 0.92 (s, 3H, 18-CH_3).

(f) Synthesis of Methyl 3-Hydroxyestra-1,3,5(10)-trien-17-one-2-carboxylate (**64**):

To a solution of methyl 3-acetoxyestra-1,3,5(10)-trien-17-one-2-carboxylate (**63**, 0.746 g, 2.0 mmol) in THF (10 mL) and MeOH (15 mL) was added sodium hydride (0.240 g of a mineral oil dispersion, 60%, 6.0 mmol) at 0°C. The reaction mixture was stirred for 30 min, and quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1, v/v) to afford 0.572 g of **64** (87% yield) mp: 178-179°C.

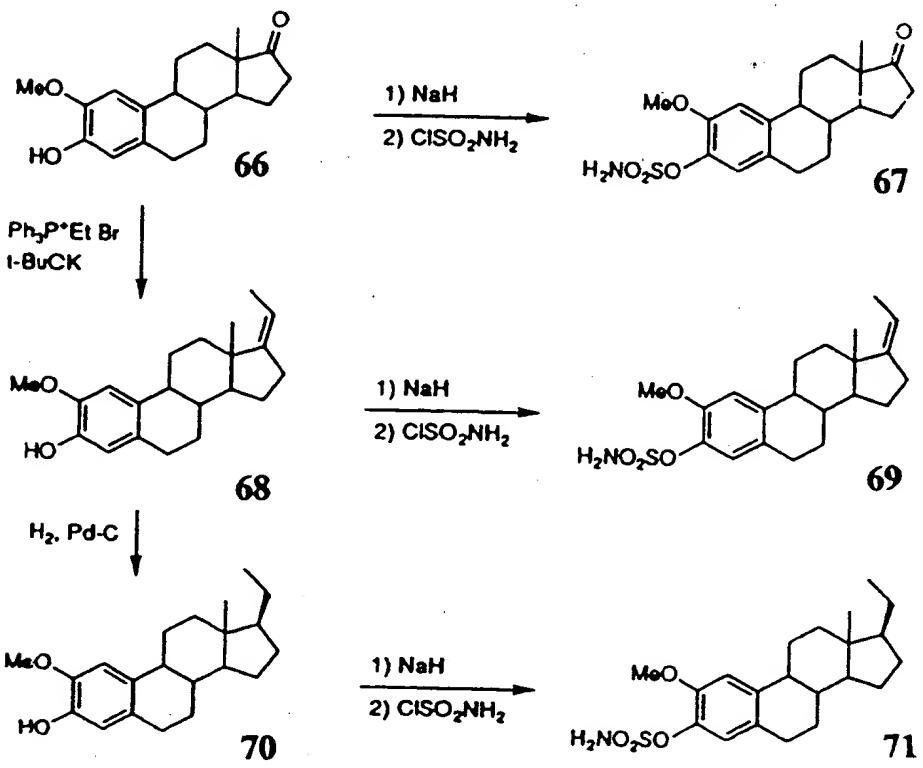
^{1}H NMR: d 10.50 (s, 1H, -OH), 7.73 (s, 1H, aromatic), 6.72 (s, 1H, aromatic), 3.93 (s, 3H, -$COOCH_3$), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 328 (M^+).

(g) Synthesis of 2-Methoxycarbonylestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (**65**):

To a solution of chlorosulfonyl isocyanate (0.43 mL, 5.0 mmol) in CH_2Cl_2 (2.0 mL) was added formic acid (1.0 mL of a CH_2Cl_2 solution, 5.0 M, 5.0 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of methyl 3-hydroxyestra-1,3,5(10)-trien-17-one-2-carboxylate (**64**, 0.328 g, 1.0 mmol) in DMF (5.0 mL) and THF (2.0 mL) was added sodium hydride (0.20 g of a mineral oil dispersion, 60%, 5.0 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h at 0°C and additional 14 h at room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1~1:1, v/v) to afford 0.233 g of the starting material **64** (71% yield) and 0.055 g of **65** (14% yield) mp: 139-140°C.

^{1}H NMR: d 7.85 (s, 1H, aromatic), 7.20 (s, 1H, aromatic), 3.91 (s, 3H, -$COOCH_3$), 0.92 (s, 3H, 18-CH_3); MS (DCI): *m/z* 425 (M^++NH_4^+).

The following scheme illustrates the synthetic steps carried out in Examples 21, 22 and 23 to make the estrone sulfatase inhibitory compounds (**67**), (**69**) and (**71**):



Scheme 11

Example 21

Preparation of 2-Methoxyestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (67)

To a solution of chlorosulfonyl isocyanate (0.43 mL, 5.0 mmol) in CH_2Cl_2 (2.0 mL) was added formic acid (1.0 mL of a CH_2Cl_2 solution, 5.0 M, 5.0 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 2-methoxyestra-1,3,5(10)-trien-3-ol (**66**, 0.300 g, 1.0 mmol) in DMF (5.0 mL) was added sodium hydride (0.200 g of a mineral oil dispersion, 60%, 5.0 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl

isocyanate in formic acid was added, and stirring continued for 1 h and additional 2 h at room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (2:1→1:1, v/v) to afford 0.210 g of **67** (55% yield) mp: 176-177°C.

^{1}H NMR: d 7.06 (s, 1H, aromatic), 6.93 (s, 1H, aromatic), 5.06 (s, 2H, -NH_2), 3.88 (s, 3H, -OCH_3), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 379 (M^+); HRMS calcd. for $C_{19}H_{24}N_1O_5S_1$, 378.1375; found, 378.1368.

Example 22

Preparation of 2-Methoxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraene-3-*O*-sulfamate (**69**)

(a) Synthesis of 2-Methoxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**68**):

To a suspension of ethyltriphenylphosphonium bromide (2.14 g, 6.0 mmol) in THF (15 mL) was added potassium *tert*-butoxide (0.670 g, 6.0 mmol) and stirred for 30 min at room temperature. The reaction mixture was added 2-methoxyestra-1,3,5(10)-trien-3-ol (**66**), 0.600 g, 2.0 mmol), stirred for 6 h at reflux condition. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (15:1→10:1, v/v) to afford 0.571 g of **68** (91% yield) mp: 126-127°C.

^{1}H NMR: d 6.80 (s, 1H, aromatic), 6.64 (s, 1H, aromatic), 5.42 (s, 1H, -OH), 5.23-5.08 (m, 1H, =C<u>H</u>-CH_3), 3.86 (s, 3H, -OCH_3), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 312 (M^+).

(b) Synthesis of 2-Methoxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraene-3-*O*-sulfamate (**69**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 2-methoxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**68**, 0.156 g, 0.5 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1~3:1, v/v) to afford 0.191 g of 69 (98% yield) mp: 171-172°C.

^{1}H NMR: d 7.03 (s, 1H, aromatic), 6.94 (s, 1H, aromatic), 5.25-5.10 (m, 1H, =C<u>H</u>-CH_3), 5.00 (s, 2H. -NH_2), 3.87 (s, 3H, -OCH_3), 0.92 (s, 3H, 18-CH_3); MS (DCI): *m/z* 409 (M^++NH_4^+), 392 (M^++H).

<u>Example 23</u>

<u>Preparation of 2-Methoxy-19-norpregna-1,3,5(10)-triene-3-*O*-sulfamate (71)</u>

(a) Synthesis of 2-Methoxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**68**):

The procedure described in step (a) of Example 22 above was used to obtain **68** from **66**.

(b) Synthesis of 2-Methoxy-19-norpregna-1,3,5(10)-trien-3-ol (**70**):

To a solution of 2-methoxy-[17(20)Z]-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol (**68**, 0.312 g, 1.0 mmol) in MeOH (3.0 mL) and THF (3.0 mL) was added 10% palladium on carbon (0.150 g). The reaction mixture was stirred for 2 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column

chromatography (silica gel) using n-hexane:EtOAc (10:1, v/v) to afford 0.287 g of **70** (91% yield) mp: 124-125°C.

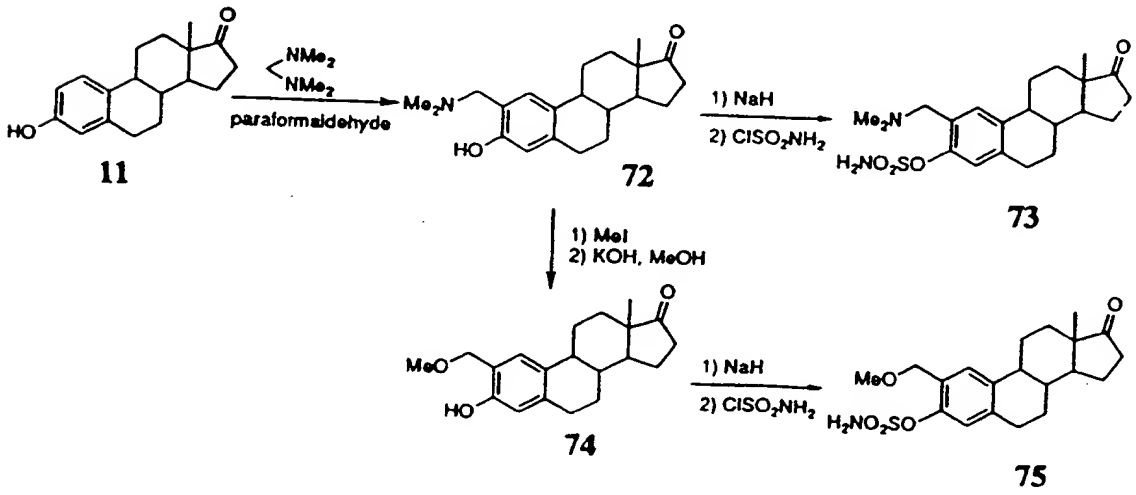
^{1}H NMR: d 6.80 (s, 1H, aromatic), 6.64 (s, 1H, aromatic), 5.41 (s, 1H, -OH), 3.86 (s, 3H, -OCH_3), 0.90 (t, 3H, 21-CH_3), 0.61 (s, 3H, 18-CH_3); MS (EI): *m/z* 314 (M^+).

(c) Synthesis of 2-Methoxy-19-norpregna-1,3,5(10)-triene-3-*O*-sulfamate (**71**):

By using the procedure described in step (b) of Example 22 above, beginning with 2-methoxy-19-norpregna-1,3,5(10)-trien-3-ol (**70**, 0.157 g, 0.5 mmol), 0.191 g of **71** (97% yield; mp: 191-192°C) was obtained after chromatography (n-hexane:EtOAc 5:1→2:1, v/v).

^{1}H NMR: d 7.03 (s, 1H, aromatic), 6.94 (s, 1H, aromatic), 4.98 (s, 2H. -NH_2), 3.87 (s, 3H, -OCH_3), 0.91 (s, 3H, 21-CH_3), 0.62 (s, 3H, 18-CH_3); MS (DCI): *m/z* 411 (M^++NH_4^+), 394 (M^++H).

The following scheme illustrates the synthetic steps carried out in Examples 24 and 25 to make the estrone sulfatase inhibitory compounds (**73**) and (**75**):



Scheme 12

Example 24

Preparation of 2-Dimethylaminomethylestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (73)

(a) Synthesis of 2-Dimethylaminomethyl-3-hydroxyestra-1,3,5(10)-trien-17-one (72):

To a suspension of estrone (**11**, 5.40 g, 20 mmol) in EtOH (100 mL) and benzene (60 mL) were added paraformaldehyde (0.600 g, 20 mmol) and *N,N,N',N'*-tetramethyldiaminomethane (5.5 mL, 40 mmol), and stirred for 20 h at 80°C. After the reaction mixture was cooled to 0°C, 5 N HCl was added. The aqueous layer was washed with Et_2O, and basified with aqueous NH_4OH. The precipitate was collected by filtration and washed with H_2O, and recrystallized from EtOH to afford 4.37 g of **72** (67% yield) mp: 172-173°C.

^{1}H NMR: d 6.86 (s, 1H, aromatic), 6.57 (s, 1H, aromatic), 3.59 (AB type, 2H, -C<u>H</u>$_2$-N(CH$_3$)$_2$), 2.31 (s, 6H, -N(CH$_3$)$_2$), 0.91 (s, 3H, 18-CH$_3$); MS (EI): *m/z* 327 (M^+).

(b) Synthesis of 2-Dimethylaminomethylestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (**73**):

To a solution of chlorosulfonyl isocyanate (0.46 mL, 5.0 mmol) in CH_2Cl_2 (2.0 mL) was added formic acid (1.0 mL of a CH_2Cl_2 solution, 5.0 M, 5.0 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 2-dimethylaminomethyl-3-hydroxyestra-1,3,5(10)-trien-17-one (**72**, 0.327 g, 1.0 mmol) in DMF (5.0 mL) was added sodium hydride (0.200 g of a mineral oil dispersion, 60%, 5.0 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added; stirring then continued for 3 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:acetone (3:1–3:2, v/v) to afford 0.093 g of the starting material **72** (28% yield) and 0.115 g of **73** (28% yield) mp: 148-149°C.

NMR: d 7.21 (s, 1H, aromatic), 7.14 (s, 1H, aromatic), 3.48 (s, 2H, $-C\underline{H}_2-N(CH_3)_2$), 2.30 (s, 6H, $-N(CH_3)_2$), 0.92 (s, 3H, 18-CH_3); MS (DCI): *m/z* 407 (M^++H).

Example 25

Preparation of 2-Methoxymethylestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (75)

(a) Synthesis of 2-Dimethylaminomethyl-3-hydroxyestra-1,3,5(10)-trien-17-one (72):

The procedure described in step (a) of Example 24 above was followed to obtain **72**.

(b) Synthesis of 3-Hydroxy-2-methoxymethylestra-1,3,5(10)-trien-17-one (74):

To a suspension of 2-dimethylaminomethyl-3-hydroxyestra-1,3,5(10)-trien-17-one (**72**, 2.0 g, 6.1 mmol) in Et_2O (200 mL) was added iodemethane (10 mL, 161 mmol) and stirred for 20 h at room temperature. The precipitate was collected by filtration and washed with Et_2O. The solid was dissolved in MeOH (50 mL) and added potassium hydroxide (5.0 g, 85%, 76 mmol), and stirred for 3 h at reflux condition. After the reaction mixture was cooled to room temperature, solvent was evaporated at reduced pressure until half volume. The reaction mixture was acidified with 5 N HCl at 0°C, and extracted with Et_2O. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:EtOAc (15:1~10:1, v/v) to afford 1.34 g of **74** (88% yield) mp: 149-151°C.

^{1}H NMR: δ 6.93 (s, 1H, aromatic), 6.63 (s, 1H, aromatic), 4.62 (AB type, 2H, $-C\underline{H}_2-OCH_3$), 3.43 (s, 3H, $-OCH_3$), 0.91 (s, 3H, 18-CH_3); MS (EI): *m/z* 314 (M^+).

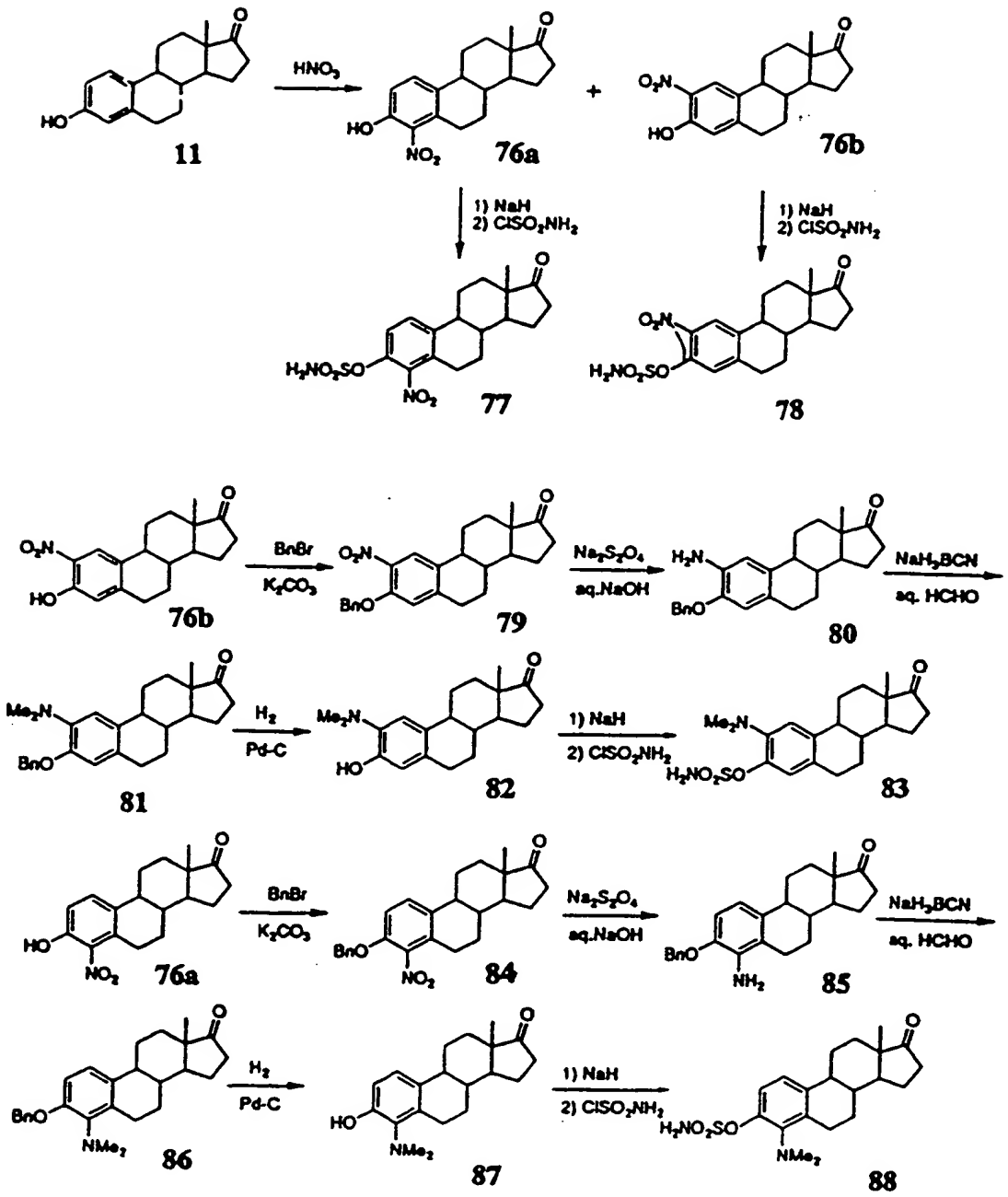
(c) Synthesis of 2-Methoxymethylestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (75):

To a solution of chlorosulfonyl isocyanate (0.46 mL, 5.0 mmol) in CH_2Cl_2 (2.0 mL) was added formic acid (1.0 mL of a CH_2Cl_2 solution, 5.0 M, 5.0 mmol) at

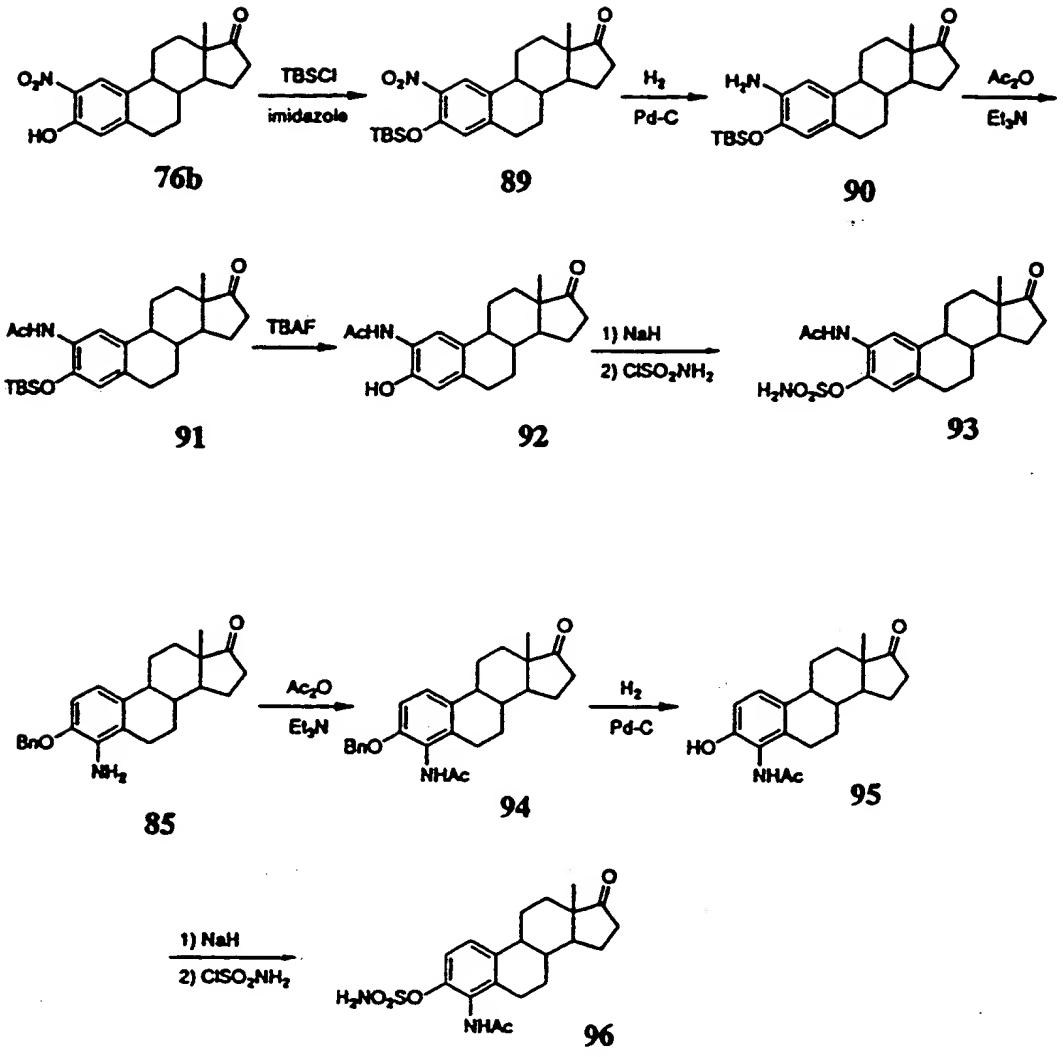
0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-hydroxy-2-methoxymethylestra-1,3,5(10)-trien-17-one (**74**, 0.314 g, 1.0 mmol) in DMF (5.0 mL) was added sodium hydride (0.200 g of a mineral oil dispersion, 60%, 5.0 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:EtOAc (10:1→3:1, v/v) to afford 0.321 g of **75** (82% yield) mp: 173-174°C.

^{1}H NMR: δ 7.30 (s, 1H, aromatic), 7.20 (s, 1H, aromatic), 5.39 (S, 2H, $-NH_2$), 4.47 (S, 2H, $-C\underline{H}_2-OCH_3$), 3.44 (s, 3H, $-OCH_3$), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 393 (M^+).

The following scheme illustrates the synthetic steps carried out in Examples 26 through 31 to make compounds (**77**), (**78**), (**83**), (**88**), (**93**), and (**96**):


HNO3
11
76a
76b
1) NaH
2) ClSO2NH2
77
78
BnBr
K2CO3
79
Na2S2O4
aq.NaOH
80
NaH3BCN
aq. HCHO
H2
Pd-C
81
82
83
84
85
86
87
88

Scheme 13



Scheme 13, continued

Example 26

Preparation of 4-Nitroestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (77)

(a) Synthesis of 3-Hydroxy-4-nitroestra-1,3,5(10)-trien-17-one (**76a**) and 3-Hydroxy-2-nitroestra-1,3,5(10)-trien-17-one (**76b**):

To a suspension of estrone (**11**, 8.11 g, 30 mmol) in acetic acid (250 mL) was heated to 120°C and cooled to 50°C. To the reaction mixture was added 70% nitric

acid (2.27 mL, 36 mmol, in an acetic acid (8.0 mL) solution) at 50°C, and stirred for 20 h at room temperature. The precipitate was filtered and washed with acetic acid, H_2O, Et_2O to afford 1.18 g of **76a** (13% yield) mp: >250°C. To the filtrate was added H_2O; it was then extracted with Et_2O. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (2:1, v/v) to afford 3.25 g of **76b** (34% yield) mp: 178-180°C.

76a: ^{1}H NMR: δ 9.46 (s, 1H, -OH), 7.52 (d, 1H, aromatic), 7.03 (d, 1H, aromatic), 0.98 (s, 3H, 18-CH_3); MS (EI): *m/z* 315 (M^+).

76b: ^{1}H NMR: δ 10.44 (s, 1H, -OH), 8.02 (s, 1H, aromatic), 6.90 (s, 1H, aromatic), 0.96 (s, 3H, 18-CH_3); MS (EI): *m/z* 315 (M^+).

(b) Synthesis of 4-Nitroestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (77):

To a solution of chlorosulfonyl isocyanate (0.43 mL, 5.0 mmol) in CH_2Cl_2 (2.0 mL) was added formic acid (1.0 mL of a CH_2Cl_2 solution, 5.0 M, 5.0 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-hydroxy-4-nitroestra-1,3,5(10)-trien-17-one (**76a**, 0.315 g, 1.0 mmol) in DMF (5.0 mL) was added sodium hydride (0.200 g of a mineral oil dispersion, 60%, 5.0 mmol) at 0°C. The reaction mixture was stirred for 1 h, and the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 4 h at room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1→3:2, v/v) to afford 0.084 g of the starting material **76a** (27% yield) and 0.243 g of 77 (62 % yield) mp: 178-180°C.

^{1}H NMR: δ 7.49 (d, 1H, aromatic), 7.43 (d, 1H, aromatic), 5.22 (s, 2H, -NH_2), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 394 (M^+).

Example 27

Preparation of 2-Nitroestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (78)

(a) Synthesis of 3-Hydroxy-4-nitroestra-1,3,5(10)-trien-17-one (**76a**) and 3-Hydroxy-2-nitroestra-1,3,5(10)-trien-17-one (**76b**):

The procedure described in step (a) of Example 26 above was used to obtain **76a** and **76b** from **11**.

(b) Synthesis of 2-Nitroestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (**78**):

The procedure described in step (b) of Example 26 above was used to obtain 0.122 g of the starting material **76b** (39% yield) and 0.165 g of **78** (42% yield; mp: 107-109°C) from 3-hydroxy-2-nitroestra-1,3,5(10)-trien-17-one (**76b**, 0.315 g, 1.0 mmol) after chromatography (n-hexane:acetone 4:1→3:2, v/v).

^{1}H NMR: δ 7.76 (s, 1H, aromatic), 7.29 (s, 1H, aromatic), 5.43 (s, 2H, -NH_2), 0.91 (s, 3H, 18-CH_3); MS (DCI): *m/z* 412 (M^++NH_4^+); HRMS calcd for $C_{18}H_{21}N_2O_6S_1$ 393.1120, found 393.1127.

Example 28

Preparation of 2-Dimethylaminoestra-1,3,5(10)-trien-17-one-3-O-sulfamate (83)

(a) Synthesis of (**76b**):

The procedure in step (a) of Example 26 above was used to obtain **76b** from **11**.

(b) Synthesis of 3-Benzyloxy-2-nitroestra-1,3,5(10)-trien-17-one (**79**):

To a solution of 3-hydroxy-2-nitroestra-1,3,5(10)-trien-17-one (**76b**, 1.58 g, 5.0 mmol) in DMF (20 mL) were added potassium carbonate (1.38 g, 10 mmol) and benzyl bromide (0.9 mL, 7.5 mmol) and stirred for 19 h at room temperature. To the reaction mixture was added saturated aqueous NH_4Cl at 0°C and extracted with $CHCl_3$. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 2.01 g of **79** (99% yield) mp: 234-235°C.

^{1}H NMR: δ 7.83 (s, 1H, aromatic), 7.55-7.30 (m, 5H, aromatic), 6.83 (s, 1H, aromatic), 5.19 (s, 2H, -OCH_2-Ph), 0.91 (s, 3H, 18-CH_3).

(c) Synthesis of 3-Benzyloxy-2-aminoestra-1,3,5(10)-trien-17-one (**80**):

To a suspension of 3-benzyloxy-2-nitroestra-1,3,5(10)-trien-17-one (**79**, 1.82 g, 4.5 mmol) in acetone (250 mL) were added 0.5 N aqueous NaOH (60 mL, 30 mmol) and sodium hydrosulfite (85%, 6.0 g) at 80°C, and stirred for 1 h. After the reaction mixture was cooled to room temperature, H_2O (150 mL) was added, acetone was removed at reduced pressure, and the remainder allowed to stand for 3 h at 0°C. The precipitate was collected by filtration and washed with H_2O to afford 1.15 g of **80** (68% yield) mp: 205-207°C.

^{1}H NMR: δ 7.60-7.30 (m, 5H, aromatic), 6.70 (s, 1H, aromatic), 6.60 (s, 1H, aromatic), 5.05 (s, 2H, -OCH_2-Ph), 0.91 (s, 3H, 18-CH_3).

(d) Synthesis of 3-Benzyloxy-2-dimethylaminoestra-1,3,5(10)-trien-17-one (**81**):

To a suspension of 3-benzyloxy-2-aminoestra-1,3,5(10)-trien-17-one (**80**, 0.751 g, 2.0 mmol) in THF (2.0 mL) and CH_3CN (10 mL) were added 37% aqueous formaldehyde (4.0 mL) and sodium cyanoborohydride (0.377 g, 6.0 mmol) and stirred for 2 h at room temperature. Additional sodium cyanoborohydride (0.377 g, 6.0 mmol) was then added to the reaction mixture, which mixture was then stirred for 20 h. Next, saturated aqueous NH_4Cl at 0°C, was added to the reaction mixture, and it was then extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:THF (5:1→3:1, v/v) to afford 0.797 g of **81** (99% yield) mp: 165-166°C.

^{1}H NMR: δ 7.55-7.25 (m, 5H, aromatic), 6.89 (s, 1H, aromatic), 6.65 (s, 1H, aromatic), 5.12 (s, 2H, -OCH_2-Ph), 2.82 (s, 6H, -$N(CH_3)_2$), 0.92 (s, 3H, 18-CH_3).

(e) Synthesis of 2-Dimethylamino-3-hydroxyestra-1,3,5(10)-trien-17-one (**82**):

To a solution of 3-benzyloxy-2-dimethylaminoestra-1,3,5(10)-trien-17-one (**81**, 0.666 g, 1.65 mmol) in THF (30 mL) was added 10% palladium on carbon

(0.200 g). The reaction mixture was stirred for 1 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1→2:1, v/v) to afford 0.462 g of **82** (89% yield) mp: 160-161°C.

^{1}H NMR: δ 7.09 (s, 1H, aromatic), 6.68 (s, 1H, aromatic), 2.64 (s, 6H, -N(CH_3)$_2$), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 313 (M^+).

(f) Synthesis of 2-Dimethylaminoestra-1,3,5(10)-trien-17-one-3-O-sulfamate (**83**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 2-dimethylamino-3-hydroxyestra-1,3,5(10)-trien-17-one (**82**, 0.157 g, 0.5 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:THF (3:1→2:1, v/v) to afford 0.082 g of the starting material **82** (52% yield) and 0.070 g of **83** (36% yield) mp: 178-179°C.

^{1}H NMR: δ 7.05 (s, 1H, aromatic), 7.02 (s, 1H, aromatic), 2.79 (s, 6H, -N(CH_3)$_2$), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 392 (M^+).

Example 29

Preparation of 4-Dimethylaminoestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (88)

(a) Synthesis of (**76a**):

The procedure described in step (a) of Example 26 above was used to obtain **76a** from **11**.

(b) Synthesis of 3-Benzyloxy-4-nitroestra-1,3,5(10)-trien-17-one (**84**):

To a solution of 3-hydroxy-4-nitroestra-1,3,5(10)-trien-17-one (**76a**, 3.15 g, 10 mmol) in DMF (40 mL) were added potassium carbonate (2.76 g, 20 mmol) and benzyl bromide (1.8 mL, 15 mmol); the mixture was then stirred for 1 h at room temperature. Saturated aqueous NH_4Cl at 0°C was then added, and the mixture was extracted with $CHCl_3$. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 3.73 g of **84** (92% yield) mp: 198-199°C.

^1H NMR: δ 7.45-7.20 (m, 6H, aromatic), 6.87 (d, 1H, aromatic), 5.15 (s, 2H, $-OCH_2$-Ph), 0.92 (s, 3H, 18-CH_3).

(c) Synthesis of 3-Benzyloxy-4-aminoestra-1,3,5(10)-trien-17-one (**85**):

To a suspension of 3-benzyloxy-4-nitroestra-1,3,5(10)-trien-17-one (**84**, 3.24 g, 8.0 mmol) in acetone (400 mL) were added 0.5 N aqueous NaOH (100 mL, 50 mmol) and sodium hydrosulfite (85%, 10 g) at 80°C; the mixture was then stirred for 1 h. After the reaction mixture was cooled to room temperature, H_2O (300 mL) was added; Acetone was then removed at reduced pressure, and the mixture allowed to stand for 3 h at 0°C. The precipitate was collected by filtration and washed with H_2O to afford 2.29 g of **85** (76% yield) mp: 219-221°C.

^1H NMR: δ 7.50-7.30 (m, 5H, aromatic), 6.76 (d, 1H, aromatic), 6.71 (d, 1H, aromatic), 5.08 (s, 2H, $-OCH_2$-Ph), 0.90 (s, 3H, 18-CH_3).

(d) Synthesis of 3-Benzyloxy-4-dimethylaminoestra-1,3,5(10)-trien-17-one (**86**):

To a suspension of 3-benzyloxy-4-aminoestra-1,3,5(10)-trien-17-one (**85**, 0.188 g, 0.5 mmol) in THF (1.0 mL) and CH_3CN (5.0 mL) were added 37% aqueous formaldehyde (1.0 mL) and sodium cyanoborohydride (0.251 g, 4.0 mmol); the mixture was then stirred for 3 h at room temperature. Additional sodium cyanoborohydride (0.251 g, 4.0 mmol) was then added, and the mixture stirred for 24 h. Saturated aqueous NH_4Cl at 0°C was added, and the mixture extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was

evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1→1:1, v/v) to afford 0.109 g of **86** (54% yield).

^{1}H NMR: δ 7.53-7.26 (m, 5H, aromatic), 7.07 (d, 1H, aromatic), 6.79 (d, 1H, aromatic), 5.07 (s, 2H, -OCH_2-Ph), 2.78 (s, 6H, -$N(CH_3)_2$), 0.90 (s, 3H, 18-CH_3).

(e) Synthesis of 4-Dimethylamino-3-hydroxyestra-1,3,5(10)-trien-17-one (**87**):

To a solution of 3-benzyloxy-4-dimethylaminoestra-1,3,5(10)-trien-17-one (**86**, 0.271 g, 0.67 mmol) in THF (10 mL) was added 10% palladium on carbon (0.200 g). The reaction mixture was stirred for 1 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1→3:1, v/v) to afford 0.171 g of **87** (81% yield) mp: 155-156°C.

^{1}H NMR: δ 7.06 (d, 1H, aromatic), 6.78 (d, 1H, aromatic), 2.84 and 2.82 (s and s, each 3H, -$N(CH_3)_2$), 0.91 (s, 3H, 18-CH_3); MS (EI): *m/z* 313 (M^+).

(f) Synthesis of 4-Dimethylaminoestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (**88**):

To a solution of chlorosulfonyl isocyanate (0.16 mL, 1.8 mmol) in CH_2Cl_2 (0.7 mL) was added formic acid (0.36 mL of a CH_2Cl_2 solution, 5.0 M, 1.8 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 4-dimethylamino-3-hydroxyestra-1,3,5(10)-trien-17-one (**87**, 0.112 g, 0.36 mmol) in DMF (2.0 mL) was added sodium hydride (0.070 g of a mineral oil dispersion, 60%, 1.7 mmol) at 0°C. The reaction mixture was stirred for 1 h, the chlorosulfonyl isocyanate in formic acid was then added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (3:1→3:2, v/v) to afford 0.069 g of the starting material **87** (61% yield) and 0.034 g of **88** (24% yield) mp: 151-152°C.

^{1}H NMR: δ 7.20 (d, 1H, aromatic), 7.09 (d, 1H, aromatic), 5.20-4.90 (br s, 2H, -NH_2), 2.84 (s, 6H, -$N(CH_3)_2$), 0.92 (s, 3H, 18-CH_3); MS (EI): *m/z* 392 (M^+).

Example 30

Preparation of 2-Acetoamideestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (93)

(a) Synthesis of (**76b**):

The procedure described in step (a) of Example 26 above was used to obtain **76b** from **11**.

(b) Synthesis of 3-*tert*-Butyldimethylsilyloxy-2-nitroestra-1,3,5(10)-trien-17-one (**89**):

To a solution of 3-hydroxy-2-nitroestra-1,3,5(10)-trien-17-one (**76b**, 1.10 g, 3.5 mmol) in DMF (10 mL) were added imidazole (0.476 g, 7.0 mmol) and *tert*-butyldimethylchlorosilane (0.690 g, 4.6 mmol) at room temperature. The reaction mixture was stirred for 1 h, and diluted with EtOAc, and washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1~2:1, v/v) to afford 1.45 g of **89** (97% yield) mp: 184-185°C.

^{1}H NMR: δ 7.76 (s, 1H, aromatic), 6.68 (s, 1H, aromatic), 1.01 (s, 9H, -$C(CH_3)_3$), 0.92 (s, 3H, 18-CH_3), 0.24 (s, 6H, -$Si(CH_3)_2$).

(c) Synthesis of 2-Amino-3-*tert*-butyldimethyl-silyloxyestra-1,3,5(10)-trien-17-one (**90**):

To a solution of 3-*tert*-butyldimethylsilyloxy-2-nitroestra-1,3,5(10)-trien-17-one (**89**, 1.29 g, 3.0 mmol) in THF (30 mL) was added 10% palladium on carbon (0.200 g). The reaction mixture was stirred for 18 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1~3:1, v/v) to afford 1.08 g of **90** (87% yield) mp: 173-174°C.

^{1}H NMR: δ 6.66 (s, 1H, aromatic), 6.45 (s, 1H, aromatic), 1.01 (s, 9H, -C(CH_3)$_3$), 0.90 (s, 3H, 18-CH_3), 0.24 (s, 6H, -Si(CH_3)$_2$).

(d) Synthesis of 2-Acetoamide-3-*tert*-butyldimethyl-silyloxyestra-1,3,5(10)-trien-17-one (**91**):

To a solution of 2-amino-3-*tert*-butyldimethyl-silyloxyestra-1,3,5(10)-trien-17-one (**90**, 0.416 g, 1.0 mmol) in CH_2Cl_2 (5.0 mL) were added triethylamine (0.34 ml, 2.5 mmol) and acetic anhydride (0.14 mL, 1.5 mmol), and stirred for 4 h at room temperature. Saturated aqueous $NaHCO_3$ were next added to the reaction mixture, which mixture was then extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:EtOAc (5:1~3:1, v/v) to afford 0.354 g of **91** (80% yield).

^{1}H NMR: δ 8.24 (s, 1H, aromatic), 7.55 (s, 1H, -NHCOCH$_3$), 6.52 (s, 1H, aromatic), 2.15 (s, 3H, -NHCOCH$_3$), 1.03 (s, 9H, -C(CH_3)$_3$), 0.90 (s, 3H, 18-CH_3), 0.25 (s, 6H, -Si(CH_3)$_2$).

(e) Synthesis of 2-Acetoamide-3-hydroxyestra-1,3,5(10)-trien-17-one (**92**):

To a solution of 2-acetoamide-3-*tert*-butyldimethylsilyloxyestra-1,3,5(10)-trien-17-one (**91**, 0.339 g, 0.77 mmol) in THF (5.0 mL) was added tetrabutylammonium fluoride (0.80 mL of a THF solution, 1.0 M, 0.80 mmol) at 0°C. The reaction mixture was stirred for 10 min, diluted with EtOAc, washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 0.228 g of **92** (91% yield) mp: >250°C.

^{1}H NMR: δ 8.62 (s, 1H, aromatic), 7.53 (s, 1H, -NHCOCH$_3$), 6.86 (s, 1H, -OH), 6.76 (s, 1H, aromatic), 2.25 (s, 3H, -NHCOCH$_3$), 0.91 (s, 3H, 18-CH_3); MS (EI): *m/z* 327 (M^+).

(f) Synthesis of 2-Acetoamideestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (**93**):

To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at

0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-hydroxy-2-acetoamideestra-1,3,5(10)-trien-17-one (**92**, 0.163 g, 0.5-mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, the chlorosulfonyl isocyanate in formic acid was then added, and stirring continued for 3 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:THF (5:1-2:1, v/v) to afford 0.130 g of **93** (64% yield) mp: 180-182°C.

^{1}H NMR: δ 7.93 (s, 1H, -N<u>H</u>COCH$_3$), 7.74 (s, 1H, aromatic), 7.12 (s, 1H, aromatic), 5.61 (s, 2H, -NH_2), 2.16 (s, 3H, -NHCOC<u>H</u>$_3$), 0.89 (s, 3H, 18-CH_3); MS (DCI): *m/z* 407 (M^++H).

<u>Example 31</u>

<u>Preparation of 4-Acetoamideestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (96)</u>

(a) Synthesis of 4-Amino-3-benzyloxyestra-1,3,5(10)-trien-17-one (**85**):

The procedure described in step (a) of Example 26 above was used to obtain **76b** from **11**.

(b) Synthesis of 3-Benzyloxy-4-aminoestra-1,3,5(10)-trien-17-one (**85**):

The procedure described in steps (b) and (c) of Example 29 above was used to obtain **85** from **76b**.

(c) Synthesis of 4-Acetoamide-3-benzyloxyestra-1,3,5(10)-trien-17-one (**94**):

To a suspension of 4-amino-3-benzyloxyestra-1,3,5(10)-trien-17-one (**85**, 0.376 g, 1.0 mmol) in CH_2Cl_2 (5.0 mL) and THF (2.0 mL) were added triethylamine (0.34 ml, 2.5 mmol) and acetic anhydride (0.14 mL, 1.5 mmol); the mixture was then stirred for 20 h at room temperature. Saturated aqueous $NaHCO_3$ was then added to the reaction mixture, which mixture was then extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried

(Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was washed with Et_2O to afford 0.338 g of **94** (81% yield) mp: 212-213°C.

^1H NMR: δ 7.47-7.30 (m, 5H, aromatic), 7.18 (d, 1H, aromatic), 6.82 (d, 1H, aromatic), 6.75 (s, 1H, -NHCOCH$_3$), 5.06 (s, 2H, -OCH$_2$Ph), 2.17 (s, 3H, -NHCOCH$_3$), 0.90 (s, 3H, 18-CH$_3$).

(d) Synthesis of 4-Acetoamide-3-hydroxyestra-1,3,5(10)-trien-17-one (**95**):

To a solution of 4-acetoamide-3-benzyloxyestra-1,3,5(10)-trien-17-one (**94**, 0.313 g, 0.75 mmol) in THF (10 mL) was added 10% palladium on carbon (0.100 g). The reaction mixture was stirred for 3 h under a hydrogen atmosphere at room temperature. After the catalyst was filtered, the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:acetone (2:1→3:2, v/v) to afford 0.250 g of **95** (100% yield) mp: 158-159°C.

^1H NMR: δ 7.17 (d, 1H, aromatic), 7.08 (s, 1H, -NHCOCH$_3$), 6.91 (d, 1H, aromatic), 2.31 (s, 3H, -NHCOCH$_3$), 0.90 (s, 3H, 18-CH$_3$); MS (EI): *m/z* 327 (M^+).

(e) Synthesis of 4-Acetoamideestra-1,3,5(10)-trien-17-one-3-*O*-sulfamate (**96**):

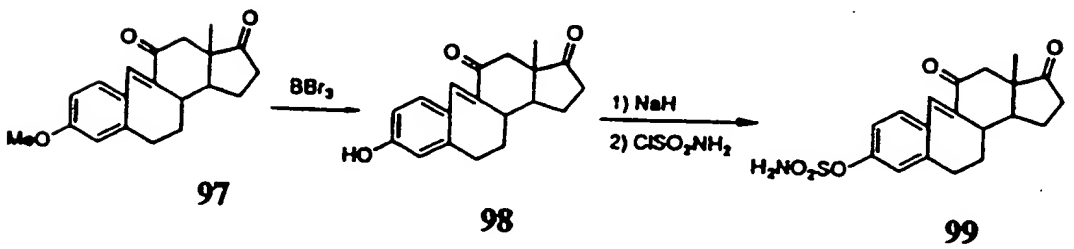
To a solution of chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) in CH_2Cl_2 (1.0 mL) was added formic acid (0.5 mL of a CH_2Cl_2 solution, 5.0 M, 2.5 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-hydroxy-4-acetoamideestra-1,3,5(10)-trien-17-one (**95**, 0.152 g, 0.46 mmol) in DMF (3.0 mL) was added sodium hydride (0.100 g of a mineral oil dispersion, 60%, 2.5 mmol) at 0°C. The reaction mixture was stirred for 1 h, the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 3 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using n-hexane:acetone (3:1→3:2, v/v) to afford 0.040 g

of the starting material **95** (27% yield) and 0.087 g of **96** (47% yield) mp: 176-178°C.

^{1}H NMR ($CDCl_3$-DMSO-*d*6): d 8.14 (d, 1H, aromatic), 7.32 (d, 1H, aromatic), 6.59 (s, 2H, -NH_2), 2.21 (s, 3H, -NHCOCH_3), 0.90 (s, 3H, 18-CH_3); MS (DCI): *m/z* 424 (M^++NH_4^+), 407 (M^++H).

Example 32

Preparation of β-Homo-9-(10→19)-abeoestra-1,3,5(10),9(19)-tetraen-11,17-dione-3-*O*-sulfamate (**99**)



97 **98** **99**

Scheme 14

(a) Synthesis of 3-Hydroxy-β-homo-9-(10→19)-abeoestra-1,3,5(10),9(19)-tetraen-11,17-dione (**98**):

To a solution of 3-methoxy-β-homo-9-(10→19)-abeoestra-1,3,5(10),9(19)-tetraen-11,17-dione (**97**, 0.931 g, 3.0 mmol) in CH_2Cl_2 (35 mL) was added boron tribromide (15 mL of a CH_2Cl_2 solution, 1.0 M, 15 mmol) at 0°C. After stirring for 3 h, additional boron tribromide (5.0 mL of a CH_2Cl_2 solution, 1.0 M, 5.0 mmol) was added. The reaction mixture was stirred for an additional 1 h, then quenched with H_2O, and then extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried (Na_2SO_4). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified

by column chromatography (silica gel) using $CHCl_3$:EtOAc (5:1, v/v) to afford 0.552 g of **98** (62% yield) mp: 225-226°C.

^1H NMR: δ 7.56-7.20 (m, 2H, aromatic), 6.80-6.70 (m, 2H, aromatic), 5.53 (s, 1H, -OH), 0.99 (s, 3H, 18-CH_3); MS (EI): *m/z* 296 (M^+).

(b) Synthesis of β-Homo-9-(10→19)-abeoestra-1,3,5(10),9(19)-tetraen-11,17-dione-3-*O*-sulfamate (**99**):

To a solution of chlorosulfonyl isocyanate (2.6 mL, 30 mmol) in CH_2Cl_2 (15 mL) was added formic acid (6.0 mL of a CH_2Cl_2 solution, 5.0 M, 30 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. To a solution of 3-hydroxy-*β*-homo-9-(10→19)-abeoestra-1,3,5(10),9(19)-tetraen-11,17-dione (**98**, 1.78 g, 6.0 mmol) in DMF (40 mL) was added sodium hydride (0.840 g of a mineral oil dispersion, 60%, 21 mmol) at 0°C. The reaction mixture was stirred for 1 h, then the chlorosulfonyl isocyanate in formic acid was added, and stirring continued for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0°C, and extracted with EtOAc. The combined organic layers were washed with H_2O, saturated aqueous NaCl, and then dried ($MgSO_4$). The desiccant was filtered and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel) using $CHCl_3$:MeOH (30:1→15:1, v/v) to afford 1.68 g of **99** (75% yield) mp: 191-192°C.

^1H NMR ($CDCl_3$-DMSO-*d6*): d 7.44-7.12 (m, 4H, aromatic), 6.94 (s, 2H, -NH_2), 0.88 (s, 3H, 18-CH_3); MS (NES): *m/z* 374 (M^+-H); HRMS calcd. for $C_{19}H_{20}N_1O_5S_1$, 374.1062; found, 374.1049.

Example 33

Biological Evaluation: Procedures and Results

A: Effects of Inhibitors on Estrone Sulfatase Activity in MCF-7 Cells

Reagents: MCF-7 human breast cancer cell line was supplied by the American Type Culture Collection, Rockville, MD. Eagle's minimum essential medium (MEM) and fetal calf serum (FCS) were purchased from Sigma chemical Company, St.

Louis, MO. [4-^{14}C]Estrone, [6,7-^{3}H]estradiol and [6,7-^{3}H](N)estrone sulfate were obtained from New England Nuclear Research Products, Boston, MA.

Procedure: The method of Duncan et al., *Cancer Res.* 53:298-303 (1993) was employed. MCF-7 cells were seeded in 60mm x 15mm culture dishes at 1 x 10^5 cells/dish and maintained in 4.0 ml of MEM containing 2 mM glutamine and 5% FCS. The cells were incubated at 37°C in an atmosphere of 5% CO_2/95% air and 100% humidity, with the medium changed every third day. when the cells reached 80% confluency, the intact monolayers were washed once with Earl's balanced salt solution and incubated in 4.0 ml of serum and phenol re-free MEM containing either the substrate (^{3}H-estrone sulfate, 7 pmol, 9 x 10^5 dpm) and inhibitor dissolved in ethanol, or ethanol alone. The final ethanol concentration was always below 1%. The incubation continued under the regular conditions for 24 h. At the end of 24 h, 2.0 ml of medium was transferred into separate tubes containing 7 x 10^3 dpm of ^{14}C-estrone. The mixture was vortexed vigorously for 60 s with 5 ml of toluene. After phase separation, 2.0 ml of the organic phase was transferred into a counting vial for scintillation counting. The amount of estrone sulfate hydrolyzed was calculated on the basis of ^{3}H counts obtained, with the added ^{14}C-estrone counts used to correct for recovery through the extraction procedure.

The cells remaining in each culture dish were washed once with saline and then scraped with 1.0 ml of 0.5 N NaOH into 10 x 75 mm tubes. The cell pellets in each tube was incubated at 50°C for 20 min to ensure that digestion was complete and all proteins had became soluble. An aliquot was then taken for protein determination by Lowry's method (Lowry et al., *J. Biol. Chem.* 193:265-275 (1951)).

The percentage of inhibition was determined by evaluating the quantity of estrone sulfate hydrolyzed with the inhibitor relative to that without the inhibitor.

As a general practice, all available inhibitors were first tested at 100 μM; the ones showing inhibitory effects at that concentration were tested again at various concentrations to obtain the IC_{50} values.

B. Uterotrophic and antiuterotrophic assays

Sprague Dawley rates are obtained from Simmonsen Laboratories, Gilroy, CA. Estradiol benzoate may be purchased from Sigma Chemical Co., St. Louis, MO.

The procedure of Wakeling et al., *Endocrinology* 99:447-453 (1983) is followed. Female Sprague Dawley rats weighing 40-50 grams are used for the experiment. In general, animals are quarantined for 3 days after arrival at the experimental site.

Rats are initially weighed and randomly divided into groups with 5 animals in each group. For the uterotrophic assay, animals are dosed once daily with various doses of test compounds in 0.1 or 1.0 ml of sterile saline via subcutaneous injection or oral gavage, respectively. For the antiuterotrophic assay, animals are dosed once daily with the same doses indicated above plus 0.5 μg/rat of estradiol benzoate alone.

The animals are treated for 7 days. On day 8, animals are weighed and then sacrificed. The uterus of each animal is removed immediately after death and weighed; fat materials are trimmed off prior to weighing.

A comparison of uterine weights from the groups receiving test compound alone with those of the vehicle control group gives the estrogenic activity. Antiestrogenic activity is obtained by comparing the uterine weights from the groups receiving test compound plus estradiol with those of the estradiol control group.

The results of the biological testing procedures used to evaluate the compounds of the invention are set forth in the following table:

Compound Number	Estrone Sulfatase Inhibitory Activity IC_{50}	Estrogenic Activity
5	250 pM	0.40
7	21 pM	0.66
10	2 nM	0
13	80 pM	0.05
15	38 pM	0.30
17	11 pM	0.55

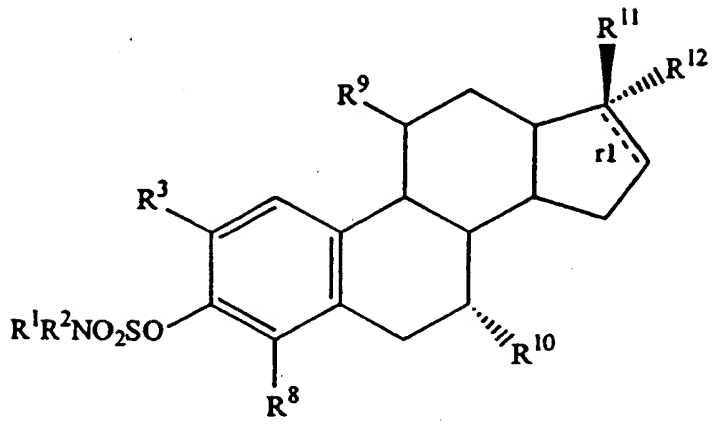
Compound Number	Estrone Sulfatase Inhibitory Activity IC_{50}	Estrogenic Activity
19	20 pM	0.54
21	34 pM	0
23	96 pM	0.02
28	27 pM	0.20
32	2.7 nM	0
34	270 pM	0
36	90 pM	0.44
39	34 nM	0
44	24 nM	0
47	23 nM	0.27
52	310 pM	0
55	26 nM	0
59	300 pM	0
65	>1 nM	0
67	16 pM	0
69	2.5 nM	0
71	1.5 nM	0
73	>1 μM	0
75	2 nM	0
77	<10 nM	0
78	7 nM	0
83	270 nM	0
88	515 nM	0
93	410 nM	0.02
96	290 nM	0
99	26 nM	0

CLAIMS

We claim:

1. A compound having the structural formula

(I)

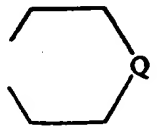


wherein:

r1 is an optional double bond;

R^1 and R^2 are selected from the group consisting of hydrogen and lower alkyl, or together form a cyclic substituent (II)

(II)



wherein Q is NH, O or CH_2;

R^3 is selected from the group consisting of hydrogen, -CN, $-NO_2$, $-COOR^4$ wherein R^4 is hydrogen or lower alkyl, $-(CH_2)_nOR^5$ and $-(CH_2)_nNR^6R^7$ wherein n is 0 to 6, R^5 is hydrogen or lower alkyl, and R^6 and R^7 are selected from the group consisting of hydrogen, lower alkyl and lower acyl, or together form the cyclic substituent (II);

R^8 is selected from the group consisting of hydrogen, $-NO_2$, and NR^6R^7;

R^9 and R^{10} are independently selected from the group consisting of hydrogen and lower alkyl;

when r1 is present, one of R^{11} and R^{12} is not present and the other is hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively; and

when r1 is not present, one of R^{11} and R^{12} is hydrogen and the other is lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively, or R^{11} and R^{12} together form =O or $=CR^{13}R^{14}$ in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, -CN, $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ and $-COOR^4$;

and pharmaceutically acceptable salts and esters thereof.

2. The compound of claim 1 wherein R^1, R^2, R^9 and R^{10} are hydrogen, and the optional double bond r1 is not present.

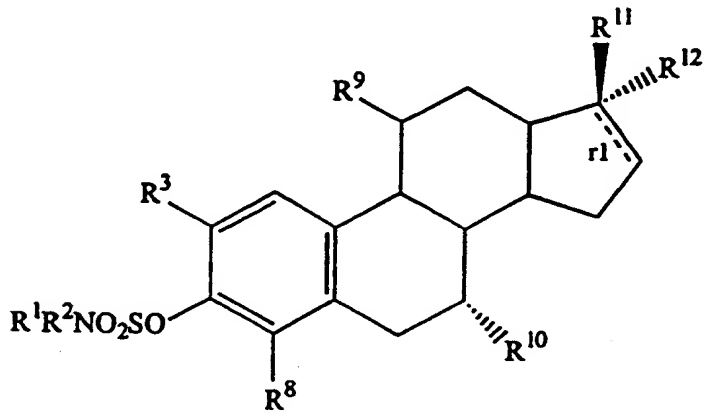
3. The compound of claim 2 wherein R^{11} and R^{12} together form =O.

4. The compound of claim 2 wherein R^{11} and R^{12} together form $=CR^{13}R^{14}$ in which one of R^{13} and R^{14} is hydrogen, or R^{13} and R^{14} are both -CN.

5. The compound of claim 2 wherein one of R^{11} and R^{12} is hydrogen and the other is $-(CH_2)_m-O(CH_2)_q-N(CH_3)_2$, m is 0 or 1, and q is 2, 3 or 4.

6. A method for inhibiting the enzymatic activity of estrone sulfatase comprising contacting the enzyme with an effective estrone sulfatase inhibiting amount of a compound having the structural formula (I)

(I)

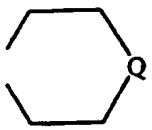


wherein:

r1 is an optional double bond;

R^1 and R^2 are selected from the group consisting of hydrogen and lower alkyl, or together form a cyclic substituent (II)

(II)



wherein Q is NH, O or CH_2;

R^3 is selected from the group consisting of hydrogen, -CN, $-NO_2$, $-COOR^4$ wherein R^4 is hydrogen or lower alkyl, $-(CH_2)_nOR^5$ and $-(CH_2)_nNR^6R^7$ wherein n is 0 to 6, R^5 is hydrogen or lower alkyl, and R^6 and R^7 are selected from the group

consisting of hydrogen, lower alkyl and lower acyl, or together form the cyclic substituent (II);

R^8 is selected from the group consisting of hydrogen, $-NO_2$, and NR^6R^7;

R^9 and R^{10} are independently selected from the group consisting of hydrogen and lower alkyl;

when r1 is present, one of R^{11} and R^{12} is not present and the other is hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively; and

when r1 is not present, one of R^{11} and R^{12} is hydrogen and the other is lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively, or R^{11} and R^{12} together form =O or $=CR^{13}R^{14}$ in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, -CN, $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ and $-COOR^4$;

or a pharmaceutically acceptable salt or ester thereof.

7. The method of claim 6 wherein, in the compound of formula (I), R^1, R^2, R^9 and R^{10} are hydrogen, and the optional double bond r1 is not present.

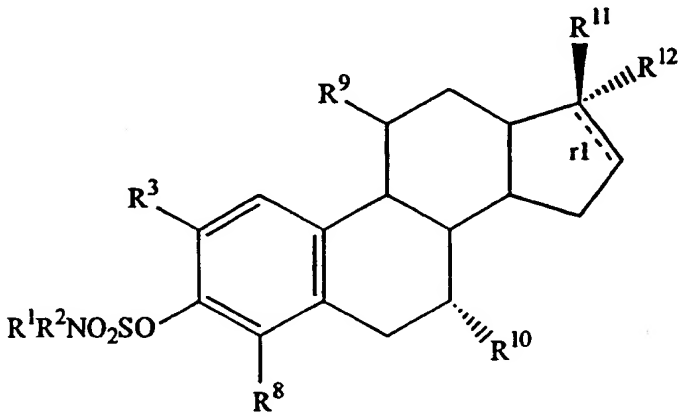
8. The method of claim 7 wherein, in the compound of formula (I), R^{11} and R^{12} together form =O.

9. The method of claim 7 wherein, in the compound of formula (I), R^{11} and R^{12} together form $=CR^{13}R^{14}$ in which one of R^{13} and R^{14} is hydrogen, or R^{13} and R^{14} are both -CN.

10. The method of claim 7 wherein, in the compound of formula I, R^{11} and R^{12} is hydrogen and the other is $-(CH_2)_m-O(CH_2)_q-N(CH_3)_2$, m is 0 or 1, and q is 2, 3 or 4.

11. A method for treating an individual with an estrogen-dependent disorder, comprising administering to the individual a pharmacologically effective amount of a compound of structural formula (I)

(I)

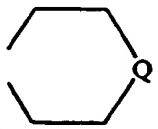


wherein:

r1 is an optional double bond;

R^1 and R^2 are selected from the group consisting of hydrogen and lower alkyl, or together form a cyclic substituent (II)

(II)



wherein Q is NH, O or CH_2;

R^3 is selected from the group consisting of hydrogen, -CN, $-NO_2$, $-COOR^4$ wherein R^4 is hydrogen or lower alkyl, $-(CH_2)_nOR^5$ and $-(CH_2)_nNR^6R^7$ wherein n is 0 to 6, R^5 is hydrogen or lower alkyl, and R^6 and R^7 are selected from the group

consisting of hydrogen, lower alkyl and lower acyl, or together form the cyclic substituent (II);

R^8 is selected from the group consisting of hydrogen, $-NO_2$, and NR^6R^7;

R^9 and R^{10} are independently selected from the group consisting of hydrogen and lower alkyl;

when r1 is present, one of R^{11} and R^{12} is not present and the other is hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively; and

when r1 is not present, one of R^{11} and R^{12} is hydrogen and the other is lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, lower acyl, lower acyloxy, or $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ wherein m and q are integers in the range of 0 to 6 and 1 to 6, respectively, or R^{11} and R^{12} together form =O or $=CR^{13}R^{14}$ in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, -CN, $-(CH_2)_m-O-(CH_2)_q-NR^6R^7$ and $-COOR^4$;

or a pharmaceutically acceptable salt or ester thereof.

12. The method of claim 11 wherein, in the compound of formula (I), R^1, R^2, R^9 and R^{10} are hydrogen, and the optional double bond r1 is not present.

13. The method of claim 12 wherein, in the compound of formula (I), R^{11} and R^{12} together form =O.

14. The method of claim 12 wherein, in the compound of formula (I), one of R^{13} and R^{14} is hydrogen, or R^{13} and R^{14} are both -CN.

15. The method of claim 12 wherein, in the compound of formula (I), one of R^{11} and R^{12} is hydrogen and the other is $-(CH_2)_m-O(CH_2)_q-N(CH_3)_2$, m is 0 or 1, and q is 2, 3 or 4.

16. A pharmaceutical composition comprising an effective estrone sulfatase inhibiting amount of the compound of claim 1 in combination with a pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising an effective estrone sulfatase inhibiting amount of the compound of claim 2 in combination with a pharmaceutically acceptable carrier.

18. A pharmaceutical composition comprising an effective estrone sulfatase inhibiting amount of the compound of claim 3 in combination with a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising an effective estrone sulfatase inhibiting amount of the compound of claim 4 in combination with a pharmaceutically acceptable carrier.

20. A pharmaceutical composition comprising an effective estrone sulfatase inhibiting amount of the compound of claim 5 in combination with a pharmaceutically acceptable carrier.